ASSESSING ANTHROPOGENIC IMPACTS ON REEF COMMUNITIES: PATTERNS, INDICATORS AND PROCESSES

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Declaration of Originality

This thesis contains no material that has been accepted for the award of any degree or diploma by the University of Tasmania or any other institution, except by way of background information and duly acknowledged in the thesis, and to the best of my knowledge and belief no material previously published or written by another person except where due acknowledgment is made in the text of the thesis.

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Abstract

Estuaries have been favoured for human settlement, and since then have slowly deteriorated as a consequence of multiple and interacting anthropogenic impacts in their unique sheltered environments. The capacity to predict which urban impacts are most severely affecting benthic communities is essential for estuarine biological conservation. Understanding urban effects and key biological responses may help to determine where impacts take place, if effects are reversible, and how pollution affects flow through the estuarine ecosystem. In this thesis, I apply observational surveys and experimental manipulation to examine patterns in sessile communities in the marine component of estuaries, and to link changes in the ecosystem to pollution sources.

In one of the first studies to consider large scale patterns of rocky reef communities across multiple estuaries, I investigated the influences of environmental and anthropogenic factors in influencing the composition, cover and dominance of macro-algae and sessile invertebrates. Reef Life Survey underwater transect protocols were conducted in three large urban estuaries. I utilised information from photo-quadrats to disentangle natural and human impact variables and describe an environmental baseline for current sessile community patterns within these ecosystems. Statistical model selection was conservative, with environmental variables entered into the models first, and then human effects accounted for. I then tested for significant patterns for functional groups. Heavy metals and proximity to ports appeared to be the major anthropogenic drivers of patterns of temperate reef sessile biota, with functional group response consistent and generalizable for two of the three estuaries. Comparison between the three capital city estuaries highlighted a clear effect of intense urbanisation and the complex nature of historical and contemporary pollution effects on biota.

To further disentangle the effects of different pollution types on sessile reef assemblages, I conducted a manipulative experiment in one of the most heavily polluted estuaries worldwide (Derwent Estuary, Hobart). I translocated healthy sessile communities grown on concrete pavers to locations adjacent to marinas, sewerage outfalls, fish farm cages, and stormwater discharges, each with associated controls. Reef communities subjected to

chronic levels of pollution in the most heavily urbanised area differed from those outside this area, with perennial Laminariales largely replaced by stress-tolerant species. Pollution types differed in their effects on transplanted communities, with marinas showing greatest negative impact, with significant losses in canopy and foliose macroalgae. Communities near fish farms, marinas and storm water drains were characterised by abundant filamentous algae. .

A concurrent experiment using bare pavers assessed the effects of the four different pollution sources on recruitment of native and non-indigenous algae and sessile invertebrates on rocky reefs in the degraded Derwent estuary over a one year period. Non-indigenous and cryptogenic species showed significantly higher cover on experimental pavers near marinas and sewerage drains compared with associated control sites. The cover of opportunistic species was significantly higher near fish farms and sewerage outfalls, and the cover of some native species was amplified at sewerage outlets relative to the control sites. Colonisation of less desirable algal communities seems to be accelerated by some urban impacts. Results suggest that careful consideration of urban drainage is required to reduce introductions of invasive algal species. Redirection of outlets offshore into better flushed areas and relocation of dense fish farm leases away from partially enclosed areas may help reduce some of the negative changes to sessile communities.

Impacts of urban pollution on benthic assemblages can be direct or indirect, through ecological interactions. I analysed invertebrate macro and mesograzer abundances from pavers used for the reef transplantation experiment to explore the potential mediation of pollution impacts through effects of pollution on mobile fauna. Log response ratios and structural equation modelling indicated that observed responses of algal groups and grazers were directly affected by pollution, rather than through trophic pathways involving interactions between these groups.

Overall, this study provides important information to improve management in estuarine systems with macroalgal-dominated reefs. Urbanisation has clearly led to large-scale decline in abundance of 'healthy' sessile benthic species, with persisting species presumably living near their tolerance limits. Current pollution loads need to be minimised if we are to maintain and restore the unique and undervalued reef communities in the increasingly urbanised environment of estuaries.

Statement of Co-Authorship

The following people and institutions contributed to the publication of work undertaken as part of this thesis:

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Chapter 1: General Introduction

Urbanisation of coastal areas has resulted in environmental changes that threaten the natural values of many estuaries (Lotze et al., 2006). Estuaries were favoured for initial European settlement in the New World because of their suitability for ports (Edgar and Barrett, 2000). These initial nodes of settlement have been foci for development (Airoldi and Beck, 2007), the wastes from which, such as sewerage, urban drainage and industrial effluent, have been released into the marine environment. Humans have also altered hydrological cycles, affecting water quality and the flux of nutrients to near shore habitats (Connell et al., 2008). Although there have been a few broad reviews of the effects of pollution on temperate estuarine habitats (e.g., Steneck et al., 2002; Thompson et al., 2002), large gaps still remain in our knowledge, particularly of species responses to pollution between and within temperate estuaries, and related to rocky reefs, rather than soft sediment habitats. These gaps are concerning because estuaries are highly diverse and productive ecosystems, yet are among the most degraded of marine ecosystems (Crain et al., 2009; Edgar et al., 2000; Roy et al., 2001). Sliding baselines and the out of sight nature of the marine environment has inhibited broad-scale quantification of these changes.

Estuaries also harbour many iconic, rare, threatened and locally endemic species. The estuaries of southeastern Tasmania support well-known examples such as weedy sea dragons (*Phyllopteryx taeniolatus*), red handfish (*Thymichthys politus*), strap weed (*Lessonia corrugata*), giant kelp (*Macrocystis pyrifera*) and spotted handfish (*Brachionichthys hirsutus*) (Whitehead et al., 2010). The majority of documented extinctions of marine species have also occurred in these environments (Edgar et al., 2000). Unfortunately, few environmental baselines of habitat-forming species exist across whole estuary gradients, or observational comparisons between regions, and even less information is available about long-term effects of pollution. This information is needed to help inform effective mitigation measures and conservation strategies.

Urban contamination: sources and biological impacts

Estuarine systems adjacent to urban centres are the receiving environment for heavy metals, organochlorines, hydrocarbons, sewage, sediments, debris, pathogens, antifoulants, stormwater and nutrients (Scanes and Philip, 1995). Each pollution type has its own potential effects on estuarine biodiversity, while presently unpredictable synergistic impacts are also likely (Airoldi et al., 2008; Crain et al., 2008). Such synergisms may relate to chemical reactions, timing of pollutant pulses and resuspension events, and vary with the accumulation, duration and magnitude of pollution impacts (Beck, 1996).

In heavily urbanised estuaries, heavy metals (copper, lead, zinc, for example) are several orders of magnitude greater than background or pre-industrial levels (Dafforn et al., 2012). These levels can be highly toxic for many species (Johnston and Roberts, 2009). Metals enter estuaries via industrial output, antifoulants paints or run-off from roads, where they are dissolved or bound to particulates, and become resident in sediments (Long, 2000). Here, metals accumulate and persist for long periods (Birch and Taylor, 1999). They can also be resuspended, which increases the availability of the metals to benthic organisms, including those living above sediments (Hill 2014). The effects of heavy metals at the organismal scale (Chapman et al., 1998) translate to impacts on biodiversity and ecosystem health (Hill et al., 2013; Johnston and Roberts, 2009). Diversity of species typically decreases with increasing metal concentrations, whilst the abundance of a few tolerant and opportunistic species can sometimes increase (Castilla, 1996). However, patterns can vary with site conditions and season (Johnston and Keough, 2005).

Industrial ports and marinas are common along estuarine shores and are a prime source of metals, such as tributyltin, other biocides (primarily copper), polychlorinated biphenyls, chromated copper arsenate, petroleum hydrocarbons and polynuclear aromatic hydrocarbons (Callier et al., 2009), primarily from antifouling paints applied to ship hulls. Antifouling paint is applied to the hulls of boats to prevent attachment and proliferation of unwanted species (Singh and Turner, 2009), but can contribute to poor water quality (Srinivasan and Swain, 2007).

The main biocide is copper, which is toxic to many marine invertebrates and algae, and indiscriminately affects many species. The application and use of antifouling paints is not heavily regulated, and leaching is likely to contribute to elevated concentrations of many chemical constituents in the sediments of more sheltered environments and the overlying water column (Turner et al., 2009). In 2008, Australian ratified the International Convention on the Control of Harmful Anti-fouling Systems on Ships, thus application, re-application, or use of harmful anti-fouling systems containing TBT is prohibited (AMSA, 2016); however TBT accumulates on the seafloor sediments and remains in high concentrations in areas of runoff from boat wash-down areas and under marinas and wharves (Scott 1993).

Despite these potential environmental threats, few studies have examined the effects of marina-related perturbations on the benthic environment (Callier et al., 2009; Turner et al., 1997). Low macrofaunal abundances (Callier et al., 2009), loss of cover and diversity of indigenous epibiota (Johnston et al., 2011), changes in macroalgal and sessile invertebrate recruitment (Rivero et al., 2013), loss of ascidians, and increase in space availability (Turner et al., 1997), have all been associated with marinas. Boat harbours and shipping activities not only represent a significant, localised source of metal contamination but also are sources of invasive species (Dafforn et al., 2008, 2012; Williams and Grosholz, 2008). Marinas may also alter water circulation, decrease current flow and change natural sedimentation rates (Turner et al., 1997). Few of these studies have assessed biota at large scales, and sessile invertebrates and algae on reef are rarely considered (but see Clarke 2015).

Urban storm water run-off is an uncontrolled and unregulated source of contamination (Roberts et al., 2007) and is a major route by which many contaminants can enter marine ecosystems (Birch and Taylor, 1999). Stormwater-drains act as conduits, collecting contaminants and releasing nitrogen-enriched run-off into coastal systems (Cox et al., 2013). The effluent may contain heavy metals, polynuclear aromatic hydrocarbons (PAHs), herbicides and other organic compounds (Eriksson et al., 2007), which are able to accumulate in a short amount of time

(Heaney et al., 1999) and could have deleterious impacts upon marine organisms. Stormwater run-off events occur sporadically, and the effects on biota may be relatively short-lived (Burton et al., 2000). However, contaminants from stormwater become resident in sediments and deposition of sediments themselves can have impacts on a broad range of estuarine species. In addition, flows may disrupt natural estuarine dispersion processes by influencing the transport of larvae of fouling species, and may influence colonization and distribution patterns of marine invaders (Pratt et al., 1981; Ruiz et al., 1999). Studies on the effects of stormwater have been predominantly concerned with the effects of contamination on epifauna (Roberts et al., 2007), and soft sediments (Carr et al., 2000). There have been no studies that have assessed the ecological impacts of stormwater upon temperate rocky reef communities over broad scales (covering estuaries in different bioregions).

Increased nutrients are changing the biota in embayments and coastal waters around the world. In many coastal areas, large amounts of nutrients from industrial effluents, agricultural runoff and intensive aquaculture (i.e. fin fish cultures) are delivered to the water column (Ajani et al., 1999; Gillibrand et al., 1996). These effluents are characterised by high concentrations of nitrogen and phosphorus (Husa et al., 2013). Urban sewage effluent may also contribute viral, bacterial and protozoan pathogens, organic substances, and heavy metals in addition to nutrients (Shah et al., 2004). The cumulative effect of these contaminants is linked to impoverished algal assemblages that have less cover and diversity than those in unpolluted areas (Littler and Murray, 1975; Martins et al., 2012).

Salmon (Salmonid species) farming is well-recognised as a source of nutrients in the water column (Troell et al., 2003), with farms typically situated in relatively unpolluted and sheltered temperate coastal waters. Waste from fish farming can cause eutrophication close to the farms, which can affect intertidal, subtidal and soft-sediment communities (Husa et al., 2013). Elevated concentrations of ammonia have been detected a few hundred metres from salmon farms in Norway (Kutti et al., 2007) and Scotland (Sanderson et al., 2008). However, the extent and effects of dissolved wastes on biota is poorly known. Other impacts from fish farms include suspended sediments, which vary according to interactions between depth, current speed, current direction, sediment type, and latitude (Kalantzi and Karakassis, 2006). Sediments from fish farms have been known, in extreme cases, to blanket benthic communities (Shah et al., 2004), smothering the substrate and inhibiting recruitment of sessile species. Unnaturally high concentrations of contaminants and deposition of organic matter can be attributed to excess feed, faecal waste generation, organic and inorganic fertilizers, liming materials, algaecides and herbicides, disinfectants, antibiotics, inducing agents, osmoregulators, pesticides and probiotics (Shahidul Islam and Tanaka, 2004), which alter oxygen concentrations.

Productive, fast-growing algal species are a common response to nutrient enrichment (Arévalo et al., 2007; Guinda et al., 2008). Increased growth and biomass of epiphytes (Rönnberg et al., 1992), including the green alga *Ulva* (Vadas et al., 2004) and filamentous green algae such as *Cladophora* (Oh et al., 2015), are associated with fish farms, mopping up excess nutrients. However, if dense blooms occur and opportunistic algae act beyond their role as nutrient sinks (i.e. strongly reduce N:P ratios; Lavery and McComb, 1991), increased organic loading may occur that, in extreme situations, can cause oxygen depletion (Best et al., 2007), which has major consequences for the entire ecosystem. Eutrophication can lead to losses of seagrass communities (Perez et al.2008), changes in species composition and metabolism, mortality of marine organisms, and large scale loss of species diversity (Bonsdorff et al., 1997; Peterson et al., 2000). The addition of limiting nutrients can also cause shifts in competitive hierarchies and promote invasion by non-native species (Williams and Grosholz, 2008).

Difficulty in assessing impacts in estuaries

Assessing the ecological impacts of pollutants in estuaries is complicated by the lack of detailed biodiversity data available before human habitation, shifting biodiversity baselines, the inherent patchiness in the distribution of habitat types, stochastic processes, and the strong spatial and temporal environmental gradients present in estuaries. The detection of change requires impacts to be distinguishable from the background of natural variability (Veríssimo et al., 2013). Thus, to maximise pollution detection, comprehensive biodiversity assessments must be over large spatial and temporal scales, detailed, across a range on habitats, and with a good understanding of environmental characteristics.

Contaminated ecosystems are predicted to be biologically impoverished. However, pollution impact theory is derived from laboratory or small-scale field studies, and few studies replicate at the level of estuary or regional level (Clark et al., 2015). We still do not understand which anthropogenic stressors are having the strongest influence on biota and the thresholds for community collapse. Most of the studies dealing with responses of organisms to human pressures in marine waters have been undertaken in soft-bottom substrata or intertidal environments and infauna (Edgar et al., 2010). Reefs are an important estuarine habitat, one that has largely been overlooked to date (but see Stuart-Smith et al. 2015, Dafforn et al. 2012, Clarke et al. 2015).

Reef in estuaries

Rocky reefs are common in temperate estuaries and are disproportionately rich in species compared with other habitats (Oh et al., 2015). Reef areas also have high conservation value and support highly productive and diverse invertebrate, fish and algal assemblages (Edgar, 2008), which supply important ecosystem services (Vitousek et al., 2007). This critical habitat is arguably the most biodiverse in estuaries (Oh et al. 2015) where concentrations of rare species are undergoing exceptional loss of habitat (Edgar et al. 2005), yet little is known on the overall scale and nature of urban influences on assemblages.

Macroalgae are a key component of shallow temperate reefs, providing habitat, food and oxygen for a wide variety of animals, supporting high levels of biodiversity and endemism (Kerswell, 2006; Phillips, 2001). Sessile reef biota provide key ecosystem services such as nutrient cycling and water filtration. They can also act as a nursery habitat for economically-important species of fish (Morrisey, 1995). A diverse array of sessile filter-feeding invertebrates, such as bryozoans, sponges, ascidians and polychaetes, are important components of the rocky reef community. Southern

Australian sessile biota is considered diverse by global standards (Womersley 1994, 1996, Womersely & Wollaston 1998, Womersley 2003, Phillips & Blackshaw 2011), perhaps due to the lack of Pleistocene extinctions (Lüning, 1990) and the long isolation of the Australian continent (Lüning, 1990). Regardless, this ecosystem remains poorly known, as indicated by the recent discovery in an urbanised estuary in this region of the first new order of algae to be described in 50 years (Scott, 2012).

Macroalgal Indicators

Given their importance on temperate reefs, their susceptibility to anthropogenic impacts and their sedentary nature, sessile communities are regarded as important bioindicators of environmental disturbance (Juanes et al., 2008). While some taxa integrate the outcomes of past and present water quality and conditions (Gorostiaga and Díez, 1996), opportunistic algal species are useful for assessing more recent conditions. They can quickly exploit new resources (such as nitrate availability) or ecological niches as they become available facilitated by their rapid growth rate, short life spans, early reproduction, high reproductive rates and small size (Balata et al., 2007; Littler and Littler, 1980; Valiela et al., 1997). Many of these species are tolerant to sedimentation (Eriksson and Johansson, 2005) and urban pollution (Gorgula and Connell, 2004). In temperate waters, annual species, such as *Ulva*, *Cladophora* and *Chaetomorpha* (Lavery and McComb, 1991; Mann, 1973), are broadly regarded as opportunistic. In Northern Chile, one opportunistic species, *Ulva compressa,* was tolerant to high levels of copper pollution (Castilla, 1996), where almost all other species of invertebrates and algae disappeared. Similarly, turf communities are tolerant to environmental stresses (Littler and Littler, 1980), including grazing (Hay, 1981), sedimentation (Airoldi 1998), and physical disturbance (Sousa, 1980). In some cases, over-growth by opportunistic algae is associated with a decrease in species richness and cover of canopy-forming perennials (Wells et al., 2007). Thus, these opportunistic macroalgae may act as early warning signals of pollution.

Perennial species can also indicate impacts of poor water quality over longer time periods. They are longer lived, grow slowly, reproduce later and invest more energy into defensive mechanisms (Littler and Littler, 1980). Over the centuries, widespread fragmentation and decline of canopy-forming algae has occurred in many urban coastal waters (Airoldi and Beck, 2007; Connell et al., 2008; Díez et al., 2014). Seven species of *Cystoseira* are now regionally extinct in the Mediterranean Sea (Thibaut et al., 2005), and canopy-forming algae in Australia (Connell et al., 2008) and America (Steneck et al., 2002) have declined along urban coasts. Algal beds can be replaced by sea urchins barrens or turf-dominated communities as a consequence of local anthropogenic stressors (Lotze et al., 2006). Furthermore, loss of important habitat-forming species can have major ecosystem-level consequences on local productivity and biodiversity, involving reduction in breeding habitat and refugia for fish and invertebrates of commercial importance (Hughes et al., 2003). Thus, early detection of human-induced change in estuarine ecosystems is important to mitigate long term detrimental effects on a wide range of sessile and mobile species that rely on macroalgal structure for food and habitat.

Restoration

Loss of habitat-forming species is a critical step in an increasingly common transition between complex, productive communities, to simpler, less productive and depauperate communities (Folke et al., 2004). Once reef communities have transitioned, they may need human intervention (i.e. restoration) to return to their previous state. This phenomenon of alternative stable states is well-known in aquatic systems (Scheffer et al., 2001). While some attempts have been made to reverse the degradation of estuarine communities, largely by reducing urban pollutant inputs (Perkol-Finkel and Airoldi, 2010), we have little information on consequent recovery of benthic reef communities (Díez et al., 2013; Pinedo et al., 2013). Restoration of estuaries is complicated because of legacy effects and continued urban inputs. Once human changes have reduced ecosystem resilience, a return to ecological health may be slow (Perkol-Finkel and Airoldi, 2010).

In some situations, the fast growth-rates and short life spans of macroalgae make restoration via transplantation a viable and attractive management option (Carney et al., 2005). Some kelp restoration projects (out-planting and transplanting) have been successful. *Phyllospora* transplants survived in urban waters near Sydney in places where the species was once abundant but had disappeared in recent decades (Campbell et al., 2014). However, restoration of rocky reefs can be impeded by competitive exclusion (Gorman et al., 2009), increased herbivory due to release of grazers through overfishing of their predators (Steneck et al., 2002), persistent changes to environmental conditions, or combinations of these and other factors (Airoldi and Beck, 2007). Restoration will become increasingly important in urbanized, near-shore areas, but requires information on environmental conditions, natural nutrient loading (oligotrophic or eutrophic), other biotic interactions, new recovery techniques, and further understanding of the magnitude and effects of pollution.

Grazer interactions

Pollution and consumer interactions are recognized as important factors that can regulate the structure of plant communities. Larger grazers can alter physical structure and productivity (McCormick and Stevenson, 1991), in some situations eroding the resilience of kelp beds via direct physical effects (Wernberg et al., 2011). Mesograzers can facilitate the dominance of habitat-forming macrophytes by grazing competitively-superior epiphytic algae (Whalen et al., 2013), but are sensitive to pollution impacts (De-la-Ossa-Carretero et al., 2012). Pollution can also influence the palatability of algae by altering the composition of species, a consequence of changed environmental conditions. Consideration of the palatability of algal communities in urban areas and the strength of interactions between pollutant, grazers, and algal groups, represents an important element in understanding urban effects and potential restoration efforts in estuarine ecosystems.

Major gaps

The dramatic loss of habitat formed by sessile communities in estuaries has been escalating (Airoldi and Beck, 2007; Lotze et al., 2006), but not many analyses isolate the specific drivers of estuarine change in a way that allows management to more effectively prioritise those drivers that are having greatest impact. Studies of the effects of human activity on estuarine ecosystems have tended to be confined to individual estuaries, rather than extend across regional or national scales (but see Clarke et al 2015, Tweedy et al., 2012), and many have focussed on single species or habitats such as seagrass beds or soft sediments (Duffy, 2006; Edgar and Barrett, 2000). Few studies have documented changes in complex macroalgal and sessile invertebrate communities on natural rocky reefs, particularly in estuaries (but see Clark et al., 2015; Oliveira and Qi, 2003). Such knowledge is essential to accurately predict recovery in perturbed areas following management intervention.

Aims

The overall aim of this thesis is to determine the broad-scale impacts of urban pollution sources on habitat-forming communities on rocky reefs in temperate Australian estuaries. Specifically I wanted to evaluate which urban impacts were most severely affecting benthic communities, if these effects were reversible through transplants, and how impacts affect algal recruitment and grazer interactions. Field surveys and manipulative experiments were used to assess both patterns of biological response and underlying mechanisms.

This thesis is structured as a series of manuscripts, with all data chapters submitted or in the process of submission for publication in peer-reviewed journals. Thus, a degree of repetition among chapters was unavoidable, particularly in the introductory material and methods. Reference lists have been amalgamated and placed at the end of the thesis.

Structure of thesis

Chapter 2 assesses spatial patterns in sessile reef communities related to the distribution of pollutant types in three major Australian urban estuaries. It uses a correlative approach to partition variation in observed sessile community structure related to environmental and pollution gradients.

Chapter 3 is an experimental assessment of the impacts of common pollution sources on relatively healthy sessile communities. This involved a transplantation experiment to quantify and disentangle effects of marinas, sewerage outfalls, fish farm cages and stormwater discharges on natural sessile communities grown on concrete pavers.

Chapter 4 investigates the effects of four different pollution types on recruitment of macroalgae and sessile invertebrates in a degraded south-eastern Australian estuary. The response was gauged by recruitment to panels near pollution sources compared with associated control sites.

Chapter 5 investigates the interaction of macroalgae, grazing mobile invertebrates and cryptic fishes in an impacted estuary, bringing together the data from the previous experiment with new data on crypto-, macro-, and meso-herbivores.

Chapter 6 ties the data chapters together and provides a general discussion of the implications of this research.

Chapter 2. Effects of urbanisation on macroalgal and sessile invertebrate communities in three major Australian estuaries

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Abstract

We investigated the influences of environmental factors (depth, wave exposure and distance to estuary mouth) and anthropogenic factors (heavy metals, land-use, distance to nearest port/industry, and urban population density) in influencing the composition, cover and dominance of macro-algae and sessile invertebrates on rocky reefs in three south-eastern Australian capital city estuaries. Heavy metals and proximity to ports appeared to be the major anthropogenic drivers of patterns of temperate reef sessile biota after accounting for natural environmental gradients. The densities of laminarian, fucoid, brown and red foliose algae were negatively correlated with heavy metals, both in Port Phillip Bay (Melbourne) and the Derwent (Hobart), while turf, filamentous algae and some invertebrates were favoured. Sydney Harbour showed the opposite trend, with the laminarian kelp *Ecklonia radiata* most abundant near the main shipping port, whilst urchins possibly amplified loss of kelp canopy in the lower estuary. Identifying drivers of benthic communities represents a key step for effective conservation management, particularly for estuaries affected by multiple anthropogenic impacts.

Keywords: *Ecklonia radiata*, estuarine ecology, heavy metal pollution, laminarian kelp, urban impacts

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Introduction

In many regions of the world, estuaries have been, and continue to be, the focus of urbanisation, which places pressures on adjacent ecosystems (Halpern et al., 2007; Hughes et al., 2003; Vitousek et al., 2007). Estuarine shores were the first coastal sites to be settled and continue to support large human populations, leading to the contention that they are the most degraded of all marine environments (Crain et al., 2009; Roy et al., 2001). Estuarine systems adjacent to urban centres are subject to a number of contaminant releases derived from a range of urban sources, including through marinas, ports, other waterside industry, stormwater flow off urban landscapes, and through sewerage systems (Birch and Rochford, 2010a). Each pollution type has the potential to affect estuarine biodiversity differently, while synergistic and unpredictable impacts are also possible (Airoldi et al., 2008). However, assessing and understanding anthropogenic change is a challenging task, in part because detection of such change requires it to be above and beyond noise from the background of natural variability, which is naturally high in the estuarine environment (Veríssimo et al., 2013).

Assessment of responses of natural communities to human pressures in estuaries is further complicated by: the lack of detailed biodiversity data available before human habitation; shifting biodiversity baselines; the inherent patchiness in the distribution of habitat types including reefs; stochastic processes; and the large spatial and temporal gradients in estuaries. Given the lack of historical baselines and the accelerating rate of coastal development, extant biodiversity patterns within estuaries urgently need to be described, and the effects of contaminants identified, to assist urban planning and pollution management practices.

Studies on human activities in estuaries have primarily focussed on soft sediment infauna or intertidal environments, where a range of impacts have been identified. These include a loss of native species (Piola and Johnston, 2008a), increase in opportunistic species (Russell et al., 2005), reduced ecosystem resilience (Chapin et al., 2000), and an increase in susceptibility to invasion from exotic species (Williams and Grosholz, 2008b), with consequent simplification of community structure.

Pollution can lead to an eventual increase of taxa with fast reproductive strategies and greater tolerance to contaminants.

While some studies have looked at impacts in single estuaries (Courtenay et al., 2005; Oliveira and Qi, 2003), very few studies have considered impacts across multiple estuaries or large areas, although such studies are needed for conclusions to have general application (Edgar and Barrett, 2000). In addition, rocky reef habitats, which are also common within estuaries, have received much less attention (but see Stuart-Smith et al. 2015, Johnston & Roberts 2009). In relatively undisturbed estuaries, they contain some of the most diverse and productive marine plant and animal communities in temperate waters (Edgar et al., 2000). They support fishes, invertebrates, macroalgae and other biodiversity elements (Harley et al., 2012) that underpin a range of ecosystem functions, including provision of habitat and food for estuarine animals, and role as nursery locations for commercially important species (Edgar et al., 2004; Pecl et al. 2014). Rocky reefs in estuaries are usually located in shallow waters close to shore, and are often poorly flushed. These two features make them more susceptible to prolonged contamination from urban pollution than wave exposed reefs.

Macroalgae are a key component of shallow estuarine rocky reef ecosystems, with the capacity to act as bioindicators (Newton et al. 2014). The sedentary nature of macroalgal assemblages means that they can sometimes reflect past as well as present conditions (Gorostiaga and Díez, 1996), thereby providing potential indicators of ecosystem health, pollutant loadings, and integrative stressor effects. Studies in coastal environments have shown that macroalgae can respond to specific contaminants, changing their nutrient uptake, growth, and carbon storage rates (Fujita, 1985). Widespread loss of slow growing canopy-forming algae has occurred in association with declining water quality on rocky coastlines (Connell et al., 2008). Large brown algal species in the orders Laminariales (kelps) and Fucales (fuciods) near urbanised temperate coasts have declined in many regions (Gorman et al., 2009; Phillips and Blackshaw, 2011; Sales and Ballesteros, 2009; Thibaut et al., 2005), often with a concurrent increase in densities of invertebrate grazers, sea urchin barrens, or turf-dominated communities (Gorman and Connell, 2009; Ling, 2008). These changes often go unnoticed and significantly affect the structure and functioning of subtidal reef systems.

Knowledge of the specific drivers of ecological community change is critical for identifying future directions in sustainable management (Airoldi and Beck, 2007; Dayton et al., 1998). Variable patterns of disturbance can lead to diverse assemblages of organisms or, alternatively, to extensive dominance by a few species, depending on adaptive, competitive, and reproductive characteristics (Airoldi et al., 2008). Fast growing ephemeral algae such as species of *Ulva* (Mann, 1973) and *Chaetomorpha* (Juanes et al., 2008) have been used as bio-indicators of ecosystem changes from pristine to degraded (Newton et al., 2014). Yet, we do not know if trends in population and community response to pollution impacts are consistent across large scales.

Here, we consider the magnitude and nature of pollution effects across estuaries in three major Australian capital city estuaries, spanning 10 degrees of latitude. We document spatial patterns of macroalgal and sessile invertebrate cover on rocky reefs ecosystems in Sydney Harbour, Port Phillip Bay (Melbourne city) and the Derwent Estuary (Hobart city) in relation to the distribution of point sources of pollution and anthropogenic disturbance. This geographic spread allows us to examine if there are generalities across estuaries, whilst describing baseline data on the state of ecosystems. Our study of sessile organisms follows an investigation of fishes and mobile invertebrates in the three estuaries, which found impacted reefs in urbanised embayments were characterised by smaller, faster growing species, reduced fish biomass and richness, and reduced mobile invertebrate abundance and richness (Stuart-Smith et al. 2015). We hypothesise that: (1) urban stressors affect rocky reef sessile communities; (2) the structural diversity of sessile communities will vary with distance from pollutant sources and the degree of other disturbance, with the cover of large perennial macroalgae lowest at the most proximal sites; and (3) particular taxa and functional groups consistently characterise pollution impacts from different sources.

Methods

Study sites

The effect of pollution on estuarine macroalgae was assessed in the Derwent Estuary, Port Philip Bay and Sydney Harbour, adjacent to the Australian State capital cities, Hobart, Melbourne and Sydney, respectively. These temperate estuaries all have a legacy of heavy metal pollution from port and industrial activity, and ongoing storm water run-off and urban discharges (Birch 2000). All are drowned river valleys with algal-dominated rocky reefs, small (1 – 2 m) tidal ranges, and generally well-mixed saline conditions near the surface (Birch and Rochford, 2010a; Edgar et al., 2000; Hewitt et al., 2004a).

Derwent Estuary

The Derwent Estuary in Tasmania is a cool temperate estuary (Figure 1). While surface salinity from the centre of Hobart to the estuary mouth ranges from 27-35 ppt, salinity at the depth of all sites examined is close to that of seawater (35 ppt) (Whitehead et al., 2010). Average water depths in the lower estuary are 10 m - 20 m, reaching a maximum depth of ~44 m (Whitehead et al., 2010). Average flushing time for the Derwent Estuary is ~15 days (Whitehead et al., 2010). Mean sea surface temperature ranges from 12 - 17 ^{o}C, with slightly greater extremes at depths <2 m.

The shores of the Derwent Estuary are heavily urbanised, with an adjacent population of 212,000 (Whitehead et al., 2010). Sediments contain a legacy of metal contamination derived from a zinc smelter (established in 1917) and a paper mill (established in 1941) (Coughanowr & Whitehead 2013), with highly contaminated sediments extending for 40 km distance along the estuary. Contemporary impact sources include the port facilities, which are concentrated near central Hobart, and numerous effluent outlets for discharge of sewage and urban run-off throughout the estuary. The broader catchment has been heavily modified, with over 75% of the natural vegetation cleared (Butler, 2006). Agriculture and forestry have directly degraded water quality (Butler, 2006), and riverine input has been significantly modified by 10 dams and over 20 man-made upstream lakes (Whitehead et al., 2010). The volume reduction in riverine input (Davies and Kalish 1994) may cause stressor interactions with pollution/other anthropogenic stressors, however are not addressed here. Inputs from sewage treatment plants result in highest nutrient levels at mid-estuary sites, in sheltered bays, and at depth (Edgar and Barrett, 2000; Whitehead et al., 2010).

Port Phillip Bay

Port Phillip Bay (PPB) has two major cities on its banks – Melbourne and Geelong, with a total adjacent human population of approximately 3.5 million. The Yarra River provides the main source of fresh water input, but the majority of the bay remains as saline as the seawater outside the Bay, sometimes higher (salinity > 32.0 ppt) (Lee et al., 2012). Water residence time for PPB is 10–16 months (Hewitt et al., 2004b), with dominant westerly winds and tides affecting the general circulation pattern of water movement. Water temperatures typically range between 10 and 22 ^{o}C (Melbourne Water, 2012). Industrial discharges and emissions have contributed heavy metals to the bay since Melbourne's colonization in 1835 (O'leary et al., 1999). Current anthropogenic disturbances include other inorganic and organic pollutants from rivers, creeks and drains, and a major sewage treatment plant, which represents a further source of heavy metals (Fabris et al., 1999, O'leary et al., 1999). Other impacts include stormwater (Lemming et al., 1998), fishing, marinas, and resuspension of sediments by channel dredging (Hart 2008).

Sydney Harbour

Home to the central business district (CBD) of Australia's largest city (4 million people), Sydney Harbour is one of the most modified estuaries in the world (Hedge et al., 2014). The Parramatta River, Lane Cove River and Middle Harbour are major tributaries joining the main channel. Salinity in the harbour is generally similar to the ocean (35 ppt), but after rain may decline in the top layer (Hedge et al., 2014). Water temperature ranges from 16 to 25 ^{o}C. Since initial European settlement in 1803, approximately 22 % of the total 50 km^2 area of the estuary has been reclaimed by infill for industrial, recreational and residential uses (Hedge et al., 2014). Past industrial practice has resulted in large accumulations of legacy chemicals in estuarine sediments (Birch and Taylor, 1999). The establishment of a large shipping industry has also generated extensive amounts of artificial substrata (wharves, piers and floating pontoons) (Klein et al., 2011), and numerous stormwater discharges (Birth and Rochford 2010) are located along the shores and throughout the local catchment (the surface of which is 90% urbanised). Water circulation patterns vary in the estuary depending on the wind direction, which contributes to a difference in harbour retention and flushing (Hedge et al. 2014). The have been no circulation modelling studies of Sydney Harbour that fully investigate the interactions between the EAC offshore, coastal waters and the circulation within the Sydney Harbour estuary (Hedge et al. 2014).

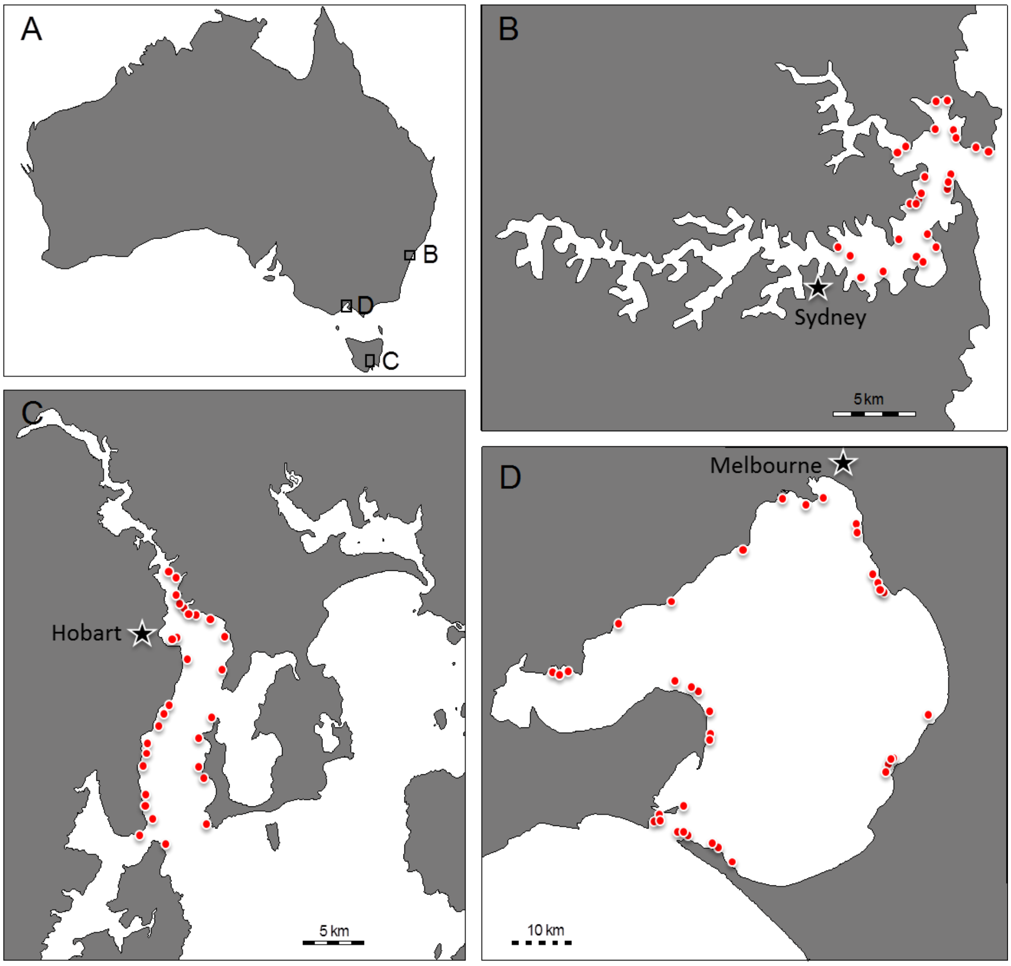

Figure 1. Maps of study estuaries and sites A: Australia, B: Sydney Harbour; C: Derwent Estuary; D: Port Phillip Bay, showing the distribution of study sites. Stars represent locations of central business districts.

Field observations

Rocky reefs in the three estuaries were surveyed in the austral summers of 2009 and 2010. A total of 36 sites were surveyed in the Derwent Estuary (Hobart), 41 in Port Phillip Bay (Melbourne) and 25 in Sydney Harbour (Fig. 1). One to six 50-m long transects were censused on natural reef at each site between 2 m and 10 m water depth. Reef Life Survey (RLS) methods were used to estimate densities of fishes, invertebrates and macro-algae, as described in Edgar and Stuart-Smith (2014) and in detail in an online methods manual (http://reeflifesurvey.com/wp-content/uploads/2015/07/NEW-Methods-Manual_150815.pdf). For sessile organisms, photo- quadrats (~0.3 x 0.3 m) were taken by divers from a height of ~50 cm every 2.5 m along each 50 m transect line.

Data analysis

Derivation of variables

The percentage cover of macro-algae, sponges, bryozoans, cnidarians, ascidians and other sessile organisms was quantified in photo-quadrats using Coral Point Count (CPCe) (Kohler and Gill, 2006). The taxon beneath each of 56 points overlaid in a grid on each image was identified to species level where possible, or to the highest practical taxonomic resolution otherwise. Some taxa could not be reliably identified and were classified into functional groups, such as 'red filamentous algae'. All points on sand, drift algae, transect tape and heavy shadow were excluded from analyses, with the percent cover for each taxon then based on the remaining points scored on all images and all transects at a site.

Values for six variables representing urban impacts, local heavy metal pollution, shipping ports, proximity to sewerage treatment plants and moored boats, land use, and local human population densities and three variables representing the natural environment, location in the estuary, wave exposure and depth were compiled for each site in each estuary (Table 1). The sources and rationale for inclusion of each variable is provides in Table 1 (and some also in Stuart-Smith et al 2015). Heavy metal data were included in different forms based on data availability. In Hobart, total heavy metals (mg/kg) were available for sediment samples. In Melbourne, they were based on water column samples (total metals in µg/L). Both data sets were collected using the same method. Pollution data were aligned with the survey sites and were derived from the closest heavy metal measurement site. In Sydney Harbour, heavy metal measurements were not available for all sites surveyed, thus we applied a three-level categorical index. Site were scored within regions known to have high (n=3), medium (n=13) or low (n=6) heavy metal loadings (Birch and Taylor 1999, Stuart-Smith et al 2015). The distance to the nearest sewage treatment plant was only obtained for sites in the Derwent Estuary, as sewerage outfalls are outside the harbour in Sydney, and too few sites were surveyed close to sewerage outfalls in Port Phillip Bay.

Variables were transformed where necessary; wave exposure was fourth root transformed, whilst distance to port, distance to estuary mouth, and urban density were log transformed (Anderson et al., 2008).

Statistical analyses

Multivariate analyses were undertaken in Primer with PERMANOVA+ (Anderson et al., 2008), with taxon cover data square root transformed. A global non-metric multidimensional scaling (MDS) ordination based on the Bray-Curtis dissimilarity matrix containing data from all three estuaries was used to first visualise overall similarities and patterns in cover. Permdisp was used to assess differences in community structure between regions. Separate DISTLM models were run for each estuary. DISTLM was used to assess the relative importance of environmental and urban impact variables for each of the three estuaries. The best subsets model selection routine based on 9999 permutations in PERMANOVA+ was used to refine the pollutant sample variables for subsequent analysis in DISTLM. This indicated that the influences of natural environmental gradients (depth, exposure, and distance to mouth) on communities need to be taken into account, thus we added these variables first to the DISTLM models, as forced inclusions. The pollution variables were then added to the model in a stepwise procedure using adjusted R^2 as the selection criterion and with P values ($\alpha = 0.05$) calculated from 9999 permutations of the residuals, providing a conservative test of the additional effects of variables related to pollutants.

The relationships between common taxa and functional groups and the strongest predictor variable in each estuary were assessed using Spearmans Rank correlation coefficient. Functional groups were chosen on the basis of their structural dominance and hypothesised response to the pollution types. The groups were Fucales, Laminariales, *Caulerpa* spp., turf algae, geniculate coralline algae, seagrass, sponges, brown, red, and green foliose algae and filamentous algae.

Table 1. Environmental and urban variable definitions.

Estuary	Variable	Abbrev.	Data Source	Origin	Proxy for
	Environmental				
DER, PPB, SYD	Location in Estuary	Mouth	Google Earth	Shortest distance (km) by water between sites and line drawn between the 'heads', where the estuary expands into open coast	Environmental gradients in a range of unmeasured factors, including intensity of low salinity pulses, current velocity, and channel width
DER, PPB, SYD	Wave exposure	Expos	Fetch model (Hill et al. 2010)	Site-specific exposure index (0-1) from cartographic fetch model	Influence of wave exposure in structuring reef communities
DER, PPB, SYD	Depth	Depth	Data measured at site	Average depth (m) of transects at site	Depth-related community structure
	Urban impact				
DER, PPB, STD	Shipping port	Port	Google Earth	Shortest distance (km) by water between sites and the main city port	Physical and chemical disturbance arising from intense shipping activity, pollution from major industry, and run-off from the CBD and industrial areas
DER, PPB, SYD	Urban density	Urb Dens	Google Earth	The number of dwellings within 200 m radius of survey sites, counted using Google Earth	Unspecified urban pollution from a range of unmeasured factors, including suburban run off related to urban housing
SYD	Sediment heavy metals	Metals	Closest site for known sediment samples (Birch et al. 1999)	A category was allocated on a three-level categorical index based on known regions of sediment heavy metal distribution within the estuary	Inorganic pollution - persistent, local heavy metal loadings
PPB	Water - column heavy metals (total metals in µg/L)	Metals	Melbourne Water	Water - column samples (total metals in µg/L)	Inorganic pollution - persistent, local heavy metal loadings
DER	Sediment heavy metals (mg/kg)	Metals	Derwent Estuary Program	Measured in sediment (mg/kg)	Inorganic pollution - persistent, local heavy metal loadings
DER	Sewerage treatment plants	STP	Google Earth	Shortest distance (km) by water between sites and nearest STP outfall (DE only)	Organic pollution - significant localised nutrient inputs
DER, PPB, STD	Moored Boats	Stat Boats	Google Earth	Number of stationary boats on moorings within 200 m of survey site, counted with Google Earth	Chemical contaminants from antifouling paints, discharges from vessels
DER, PPB, STD	Land use	Land use	Google Earth	Land use type within 200 m of survey site (park area, residential suburb)	A range of unmeasured factors, including suburban run off and more natural landscape

Results

The means of predictor variables were similar across the three estuaries (Table 2). Many correlations were observed between the predictor variables, with the strongest of these being between wave exposure and distance to port. These correlations illuminate the typical estuary configuration, with ports and high heavy metal concentrations within the sheltered upper estuary. In the Derwent Estuary, distance to port was correlated with wave exposure (> -0.71) and distance to estuary mouth (-0.78), while metals were correlated with wave exposure (-0.74), port (-0.75) and distance to mouth (-0.79). Port Phillip Bay showed a strong correlation between distance to estuary mouth and distance to port (-0.77). Sydney Harbour had a high correlation (0.83) between depth and exposure. Correlated variables were unavoidable along the gradient of an estuary; thus results were interpreted carefully as it is inherently difficult to tease apart their effects. Environmental variables were added to the model first, so correlated urban impacts were only significant if they were over and above what can be co-explained by environmental factors. Highly correlated urban impacts such as metals versus distance to port/distance to mouth are interpreted together in our results.

Table. 2. Mean, standard deviation and coefficient of variation (CV) % for environmental and urban impact variables in different estuaries, calculated using untransformed data.

Variable	DER			PPB			SYD		
	Mean	SD	CV	Mean	SD	CV	Mean	SD	CV
Environmental									
Distance to Mouth (km)	13.1	7.4	56.5	19	19.4	102.1	2.7	1.6	59.3
Depth (m)	4.6	1.7	37.0	3.4	2.1	61.8	4.7	2.5	53.2
Exposure (units)	0.03	0.04	113.9	0.07	0.08	117.0	0.06	0.09	139.4
Urban Impact									
Distance to Port (km)	9.2	6.2	67.4	40.6	17.9	44.1	8.2	2.6	31.7
Urban Density (dwellings in 200 m)	7.0	11.0	157.1	3.0	7.0	233.3	5.0	8.0	160.0
Heavy metals (mg/kg)	20694.4	18742.8	90.6						
Heavy metals (µg/L)				26.5	44.3	167.0			
Heavy metal index							2.0	1.0	50.0
Distance to STP (km)	2.4	1.9	79.2						

Two hundred and sixty-three sessile taxa were recorded from photo-quadrats across the three estuaries. Most were algae. While substantial overlap was evident in benthic community composition (Fig 2), many taxa were only recorded in a single estuary. Ninety-four taxa were observed in the Derwent, one hundred and ten in Port Phillip Bay, and fifty-nine in Sydney Harbour.

Significant differences in dispersion were observed between the communities occurring in each of the three estuaries (Permdisp $p < 0.05$). Communities at the Derwent sites were more dispersed in multivariate space, as indicated by extreme positions along both nMDS axes (Fig 2). Port Phillip Bay and Sydney Harbour sites have relatively similar communities (Permdisp $p > 0.05$), with sites more clustered than those from the Derwent, albeit with some overlap.

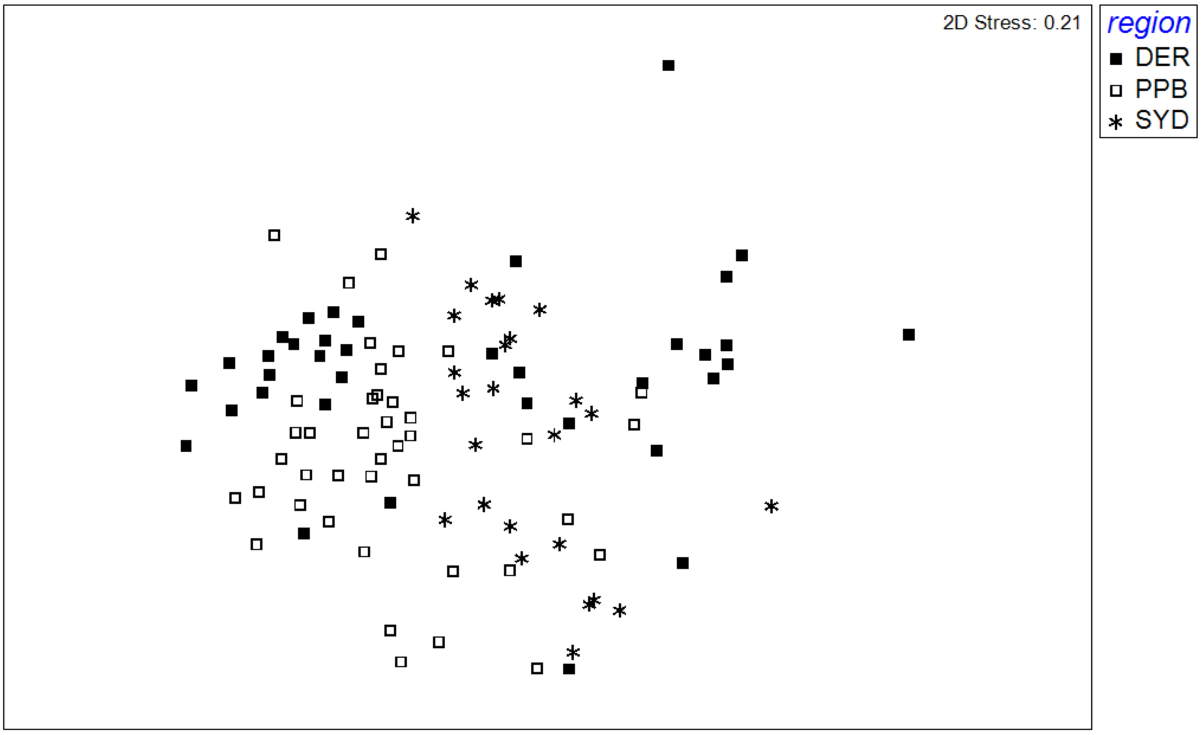

Figure 2. MDS ordination showing community-level relationships between sites for the different regions. The ordination is based on Bray Curtis similarity matrix of square root benthic community data. Symbols represent different estuaries; DER=Derwent, PPB=Port Phillip Bay, SYD=Sydney Harbour

Although many of the environmental and pollution variables were correlated, marginal DISTLM tests were also used to explore the relationship between benthic community structure and each variable on its own (Table 3), before forcing the environmental variables as a set into models, followed by stepwise addition of pollution variables. The combined variation in benthic communities described by the environmental variables alone ranged from ~17% to 30% (Table 4). The pollution variables added a further 8.7 % of variation described for the Derwent Estuary, 11.0 % for Port Phillip Bay and 5.9 % for Sydney Harbour.

Table 3. Results of marginal tests from DISTLMs. Values represent the percentage of total variation in the composition of sessile community explained by each predictor variable separately. Asterisks indicate significance level of tests; **P<0.01, *P< 0.05, NT = not tested.

Variable	DER	PPB	SYD
Environmental			
Depth	10.2**	7.6**	29.1**
Exposure	20.0**	8.7**	27.7**
Mouth	22.0**	17.1**	24.9**
Urban Impact			
Metals	26.0**	11.0**	23.9**
Port	19.9**	15.8**	17.5**
Urban Density	8.6**	6.2*	2
Boats	5.5*	3.2	1.7
STP	3.8	NT	NT
Land Use	10.2	5.2	3.7

The pollution variables explained a small but often significant proportion of variation over and above that explained by environmental factors. The sequential tests highlighted heavy metals as the one significant pollution predictor for the Derwent Estuary (Table 4). Urban density was the first pollution predictor added to the Port Phillip Bay model, followed by metals and distance to port. All were highly significant. In Sydney Harbour, distance to Port was the only significant predictor variable after taking into account environmental variables (Table 4). Heavy metals were also significant for Sydney Harbour in marginal tests, but were not significant in sequential tests after shared variation with environmental factors was accounted for.

Table 4. DISTLM results for environmental and urban pollutant variables explaining cover of sessile biota in estuaries. The numbers represent the percentage of unique variation added by each variable, with all three environmental variables forced inclusions as a set (Env). Significance is shown by **P<0.01, *P< 0.05, numbers without asterisks are non-significant, NT (not tested) indicates not available, while the blank cells were not added within the best subset by the model. The adjusted R^2 shows the explanatory power of regression models.

Estuary	Env	Metals	Stationary boats	Port	STP	Urban Density	Land Use	Adj R^2
DER	24.8**	5.0**	4.4			2.3		31.5
PPB	17.3**	4.1*	2.0	3.9*	NT	5.9**	4.8	28
SYD	30.7**	6.3	2.7	5.3*	NT		4.7	36.6

Correlations of taxa and aggregated functional groups with pollution predictor variables were often inconsistent between estuaries, however, the cover of ascidians, crustose coralline algae, other encrusting red algae, and the brown algae *Dictyopteris muelleri* and *Zonaria* spp. all decreased towards more contaminated sites (near ports and with high metal loadings) across all estuaries, while greater filamentous algae and turf cover were consistently (but not significantly) associated with higher levels of pollution (Tables 5). A number of large brown algal taxa declined towards more polluted sites in the Derwent and Port Phillip Bay, most notably *Sargassum* spp. and the major canopy-forming kelp *Ecklonia radiata* (Table 5).

When taxa were aggregated to functional groups, representatives of which were generally found within all estuaries, several patterns were evident. The strongest pollution effects were observed for the groups containing large brown macroalgae (brown foliose, Fucales and Laminariales) in the Derwent and Port Phillip (Fig 3), with a particularly high negative association between foliose brown macroalgae and heavy metal pollution in the Derwent (r = -0.78). Fewer taxa and functional groups were significantly related to the pollution gradients in Sydney Harbour. Most notably, the opposite trend was observed for the kelp *Ecklonia radiata* with greater cover at sites closer to the city port. A consistent and negative effect of pollution was evident across the three estuaries on brown algae (except Sydney), Fucales, geniculate corallines and a positive effect on turf, but these were weak or non-significant for Sydney Harbour. Other invertebrates showed apposing trends for two estuaries, in Port Phillip invertebrates increased (r = 0.56**) with heavy metals, whilst in Sydney Harbour invertebrates significantly decreased (r = - 0.64**) with distance to port district.

Table 5. Spearman rank correlations between functional groups and the strongest anthropogenic predictor variable in each estuary, statistical significant coefficients are shown by **P < 0.01, *P < 0.05, P < 0.1

Groups	Derwent Heavy metals	Port Philip Bay Heavy metals	Sydney Harbour Distance to Port
Brown foliose algae	-0.78**	-0.30	0.44**
Caulerpa spp.	-0.33*	0.23	0.07
Encrusting algae	-0.54**	-0.21	-
Filamentous algae	0.19	0.29	-0.42**
Fucales	-0.70**	-0.41**	-0.06
Geniculate coralline	-0.19	-0.33**	-0.11
Green foliose algae	0.00	-0.02	-0.03
Laminariales	-0.55**	-0.50**	0.62**
Other invertebrates	-0.08	0.56**	-0.64**
Sponges	-0.28	0.03	0.01
Red foliose algae	-0.54**	-0.31*	0.11
Seagrass	-0.21	0.20	-0.17
Turf algae	0.47**	0.22	0.10

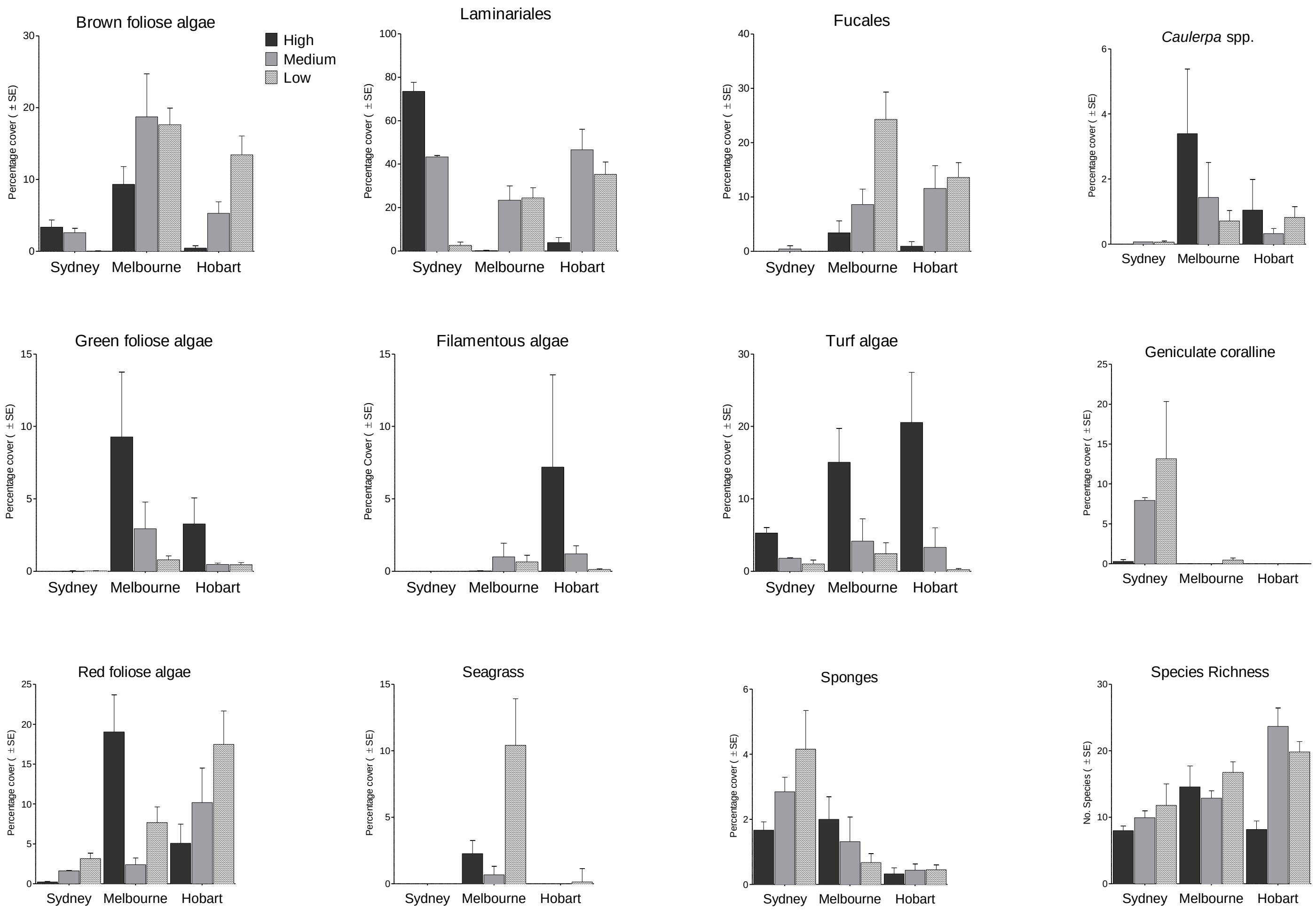

Figure 3. Average percentage cover of the benthic functional groups for high, medium and low levels of heavy metal pollution in the Derwent Estuary (Hobart), Port Phillip Bay (Melbourne) and Sydney Harbour (Sydney). The categorical values shown here were derived from Sydney metal values described in text, the Derwent sediment range from low (< 7,000 mg/kg), medium (7000-25,000 mg/kg), high (> 25,000 mg/kg) and Port Phillip Bay metals in the water column are low (< 15 µg/L), medium (15-25 µg/L), and high (>25 µg/L)

Discussion

Impacts of urbanisation and associated contaminants on sessile invertebrate and macroalgal communities were evident across three major Australian estuaries. These effects were detectable above and beyond the natural variation in environmental factors that occurs within estuaries, providing a conservative test of urban impacts, and supporting generalisation that urban development has major impacts on the benthic community (Airoldi and Beck, 2007; Crain et al., 2009; Crain et al., 2008). Further, impacts were seen on structural components of the communities. The most heavily polluted sites (close to ports and with high metal loadings) in both Port Phillip Bay and the Derwent were notably associated with much lower cover of large perennial brown algae. In the Derwent Estuary and Port Phillip Bay, impacted locations tended to support impoverished communities, consisting of smaller opportunistic species, and with habitat-forming Laminariales, Fucales, and other brown and red foliose algae largely absent, a pattern consistent with previous observations of the widespread loss of canopy-forming algae in urbanised regions (Airoldi et al., 2008; Connell et al., 2008; Strain et al., 2014). The absence of these functional groups may be considered a strong indication of urban-contaminated locations.

Urban effects were consistent across two of the three estuaries, and largely agree with expectations based on results of previous studies. In Port Philip Bay and the Derwent Estuary, large perennial macroalgae were absent near heavily polluted sites. The most influential urban-impact variable was heavy metals, a variable highly correlated with inverse distance to ports. Small, fast growing opportunistic species, such as turf and filamentous communities, were associated with high heavy metals and close proximity to ports, and turf, barnacles and other fouling invertebrates known for their ability to withstand physical and biotic stresses (Gorgula and Connell 2004) were also present in the more polluted areas. Turf communities generally show high tolerance to stressors (Littler and Littler, 1980) and recover rapidly after disturbance (Sousa, 1980). In all three estuaries, turf algae (mixed assemblages of algae <2cm in height, including filamentous species) were associated with heavily impacted sites. Urban impacts may enable turf to dominate potentially inhibiting the recruitment of canopy forming algae (Kennelly 1987). In addition, urban impacts have 'knock-on' effects on mobile species that rely on sessile organisms for food and habitat types (McKinley and Johnston 2010). This has been observed in a related study in the same three estuaries where fewer fish and invertebrate species, and reduced total fish biomass, characterise heavily impacted sites (Stuart-Smith et al., 2015b). Smaller species were found at heavily impacted sites in the Derwent Estuary and Sydney Harbour, but not Port Phillip Bay (Stuart-Smith et al., 2015b). This illustrates taxa with 'fast' life history strategies (i.e. *r*-selected) have relatively high tolerance to contaminants across the whole system.

The responses of sessile communities in Sydney Harbour were different to the other two estuaries, which has important implications for generalisation of urban impacts. Contrary to the Derwent or Port Phillip Bay, foliose algae and Laminariales in Sydney Harbour were negatively correlated with distance to ports (i.e. they increased in cover towards the more impacted areas). For the Laminariales this pattern reflected the distribution of a single species, *Ecklonia radiata.* Curiously, equivalent significant negative correlations were also evident for sessile invertebrates and filamentous algae. Assemblages in the middle estuary predominantly comprised articulated calcareous coralline algae, which are late-successional and predation-tolerant species (Littler and Littler 1984), and are expected under moderate levels of pollution (Arévalo et al., 2007). Reefs in Sydney Harbour contain higher densities and biomass of mobile fauna (Stuart-Smith et al., 2015b), in particular the grazing sea urchin *Centrostephanus rodgersii*. This species can catastrophically overgraze seaweed beds and thereby maintain 'barrens' habitat (Hill et al., 2003; Ling et al., 2015). *Centrostephanus rodgersii* numbers in NSW have apparently increased by as much as 400 % since 1962 (Young et al., 2014), whilst large urchin predators, such as blue groper (*Achoerodus viridus*) have declined. It is likely that the lack of canopy-forming kelps in the lower estuary may be influenced by high grazing pressure rather than urban inputs. In this case, biotic interactions appear to be stronger in shaping the benthic community than localised urban impacts.

Historical contamination may also influence board scale patterns of benthic reef communities. Across estuaries, contaminants associated with variation in reef sessile communities and taxa were originally primarily derived from the zinc smelter and newsprint mill in the Derwent (Townsend and Seen, 2012), and industrial discharges of cadmium, mercury and other metals in Port Phillip Bay (Phillips et al., 1992) and Sydney Harbour (Birch and Olmos, 2008). Although, dumping of toxic waste in now banned and discharges have greatly declined, the bulk of these toxins persist in the sediments, where they will remain for decades (Birch and Taylor, 1999). Of particular concern for the sessile reef biota are the close proximity to sedimentary sources of contaminants, and likelihood of re-suspension events that increase exposure of benthic flora and fauna to metals in dissolved form from sediments and through passive diffusion (Hill et al., 2009).

Although heavy metals were not found to be a significant contributing influence in the models for Sydney Harbour, they cannot be discounted as an important factor, particularly as they explained more variation in the DISTLM model than other urban impact variables. The quality and quantity of data sources differed between estuaries, and underwater ecological surveys of Sydney Harbour were not possible much further upstream of the Sydney Harbour Bridge because of high turbidity, so did not extend as far as Homebush Bay, the location known to be most heavily contaminated in Sydney (Birch and Olmos, 2008). The coarse categorical heavy metal pollution index used in Sydney Harbour had little variability, with most ecological survey sites considered to be in locations with moderate heavy metal contamination.

Distance from port was an important factor in Sydney and Melbourne. This factor is potentially a surrogate for the effects of several pollutants, including heavy metals, which probably act synergistically. Urban density also contributed significantly in the models, and represents another potential surrogate for pollutant load.

The effect of sewerage treatment plants (STP) on sessile biota was less than for heavy metals in the Derwent Estuary, despite the documented negative impacts of nutrient enrichment on biodiversity and local disappearance of large perennial algae in other regions (Gorgula and Connell, 2004; Strain et al., 2014). Excess nutrients can reduce the availability of light via phytoplankton blooms, increase turbidity, and boost epiphytic growth (Oh et al., 2015). Moreover, interactions of nutrients with other stressors can potentially lead to negative synergistic effects on growth and survival of canopy-forming algae (Strain et al., 2014), including the blocking of carbon storage (Munda and Veber, 2004). STP was not investigated for Port Phillip Bay, due to too few sites surveyed at distances expected to be influenced by the few large STPs, or Sydney, where sewage is piped outside of the Harbour.

Leached metals can also be derived from wastes associated with shipping operations, port facilities, and marinas. The prevalence of moored boats, a proxy for antifouling paints and dissolved metals particularly copper, is known to affect benthic communities (Johnston and Keough, 2005; Singh and Turner, 2009); regardless, the number of stationary boats did not detectably relate to ecological patterns in any of the three estuaries.

On the whole we found a general effect of urban impacts, and these effects were more clearly observed at the level of functional groups than for community structure based on finer taxonomic resolution. Clearest impacts were observed on red foliose algae, Laminariales, Fucales, and brown foliose algae, while turf, invertebrates and filamentous algae appear to characterise impacted areas in these estuaries.

In our study we considered a range of potential urban impacts and contaminant types. These included organic contaminants, inorganic contaminants (primarily metals), and factors that represent sources of multiple contaminant types (e.g. urban density). The reasons for this were twofold; firstly to attempt to distinguish the relative influence of different urban impacts, and secondly to assess whether particular species or functional groups consistently characterised particular types of impacts or contaminants. In reality, many potential impacts and contaminants were correlated, emphasising the difficulty in assessing relative impacts of specific sources without previous baseline data and on the basis of field observations where observed impacts represent the cumulative effects of many pressures. We were also unable to incorporate other important pollution types, such as sedimentation and turbidity. Manipulative studies are needed to disentangle these effects.

Our study indicates that the effects of urban impacts and contaminants on sessile reef fauna are detectable above the natural variability of estuarine systems. Disentangling the relative influences of impacts and contaminants in these systems is difficult; nevertheless heavy metal concentrations and distance to ports are consistently related to changing reef community structure. Responses were relatively consistent at the level of functional groups, but Sydney showed some anomalous patterns, presumably because of strong biotic interactions. Our study also provides an environmental baseline to detect change associated with urbanization within three major Australian estuaries, and describes reference conditions for testing hypotheses through manipulative experiments. Regional observations such as these are critically needed when planning effective mitigation measures and conservation strategies.

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Chapter 3. An experimental assessment of impacts of multiple pollution sources on sessile biota in a temperate urban estuary

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Abstract

Macro-algae and sessile invertebrates are the foundation of diverse rocky reef communities, yet have declined precipitously on urbanised coasts since European settlement. Responses of healthy subtidal sessile assemblages to urban impacts were evaluated in one of the most polluted estuaries in Australia. Healthy sessile communities were grown on concrete pavers and transplanted to locations adjacent to marinas, sewerage outfalls, fish farm cages, and stormwater discharges, each with associated controls. Reef communities translocated to sites adjacent to central urban pollution sources (within 5kms of Hobart) transformed to a different community than those translocated outside the most heavily impacted region, with canopy-forming algae largely replaced by stress-tolerant species. After accounting for environmental and location effects, the impacts of fish farms, marinas, and storm water drains were all characterised by increased filamentous algal cover. Marinas showed significant losses in canopy and foliose algae. Restoration of subtidal reef near highly urbanised areas and marinas may not be possible until current pollution levels are dramatically improved.

Key words: Estuarine ecology, urban impacts, indicators, macroalgae

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Introduction

Estuaries represent a focal point for human settlement and are consequently subject to greater anthropogenic disturbance compared to other coastal areas (Edgar et al., 2000). Urban contaminants inevitably impact the natural variability and composition of estuarine communities, with shifts often trending to simpler, less desirable ecological states (Folke et al., 2004). Common anthropogenic modifications to estuaries include their use as sites for ports, marinas, and aquaculture operations (Di Franco et al., 2011; Edgar et al., 2005; Singh and Turner, 2009). They also often contain numerous point sources of pollution in the form of industrial effluents (Townsend and Seen, 2012), stormwater (Birch and Rochford, 2010b; Birch and Taylor, 1999; Edgar and Barrett, 2000), and sewage (Arévalo et al., 2007; Díez et al., 2013). This intensive development modifies and contaminates water flows, impacting on benthic diversity and health (Airoldi and Beck, 2007). Gradual losses of natural condition, declines in diversity of species, and loss of ecosystem resilience have been documented in urbanised regions around the world (Edgar and Samson, 2004; Hughes et al., 2011; Lotze et al., 2006; Stuart-Smith et al., 2015b).

Despite great efforts to eliminate or reduce some pollution sources to restore water quality, macroalgal assemblage responses following pollution abatement are poorly understood (Veríssimo et al., 2013). Currently, urban impacts overlay a historic legacy of industrial contamination and urban pollution sources in combination can have synergistic and interactive effects on biota (Crain et al., 2008). In addition, estuaries contain strong physical and chemical gradients (Chester et al., 1983b) that are dynamic on relatively short temporal scales (Díez et al., 2013). Thus, quantifying current pollution effects and recovery is a challenging task, because it requires changes in community structure to exceed natural variability (Veríssimo et al., 2013).

Estuaries also often contain high species richness and endemism, particularly in Southern Australia (Scott, 2012; Womersley, 1984). Diversity is often correlated with high levels of ecosystem functioning (Cardinale et al., 2002) and resilience (Folke et al., 2004) that, when combined with endemic species, make these estuarine ecosystems of high conservation value (Edgar et al., 2000). This is particularly true of estuarine subtidal rocky reef habitats. Such reefs support a high diversity of sessile invertebrate and algal assemblages, which in turn support commercially important species, as well as providing other ecosystem services such as water filtering (Dayton, 1985; Morton and Gladstone, 2014). These reef systems, however, are usually located in shallow near-shore areas in proximity to pollution sources, which may make them particularly susceptible to change. One major concern is the fragmentation, decline and complete disappearance of canopy-forming and other seaweeds along some urban coasts (Airoldi and Beck, 2007; Connell et al., 2008; Díez et al., 2014). Canopy-forming species provide habitat, shelter and food for numerous other species in temperate coastal rocky habitats (Schmidt and Scheibling, 2007). At polluted locations, canopy algae are often replaced by tolerant and structurally less complex species, many of which trap large amounts of sediment (Gorman and Connell, 2009). Once a shift to an alternative state has occurred, it can persist even after the perturbations that caused the initial change are reduced (Perkol-Finkel and Airoldi, 2010).

Different types of urban pollution are hypothesised to have different impacts on macroalgal assemblages, depending on the nature, magnitude and duration of perturbation (Borja et al., 2010), and the reproduction, recruitment and growth characteristics of macroalgal species (Crowe et al., 2013). Sewage and finfish aquaculture increase nitrogen, organic matter, and suspended sediments, favouring competitively superior species, such as rapid colonizers, and thread-like or sheet like algae (Ajani et al., 1999; Eriksson and Johansson, 2005; Irving et al., 2009). Human-derived nutrients may enhance species richness and macroalgal production in oligotrophic systems (Littler and Murray, 1975), whilst species richness may be reduced in eutrophic conditions (Hall et al., 2000).

Increased sedimentation that arises from urban impacts can also affect the growth and reproduction of benthic organisms via physical scouring and smothering, promoting a change in species composition to faster growing or more tolerant species (Eriksson et al., 2002). Marinas are associated with a build-up of antifouling biocides and metals, such as Irgarol, copper, and tributyltin (Johnston et al., 2011; Schiff et al., 2004). Toxins may act selectively upon targeted species, but may also affect non-targeted native biota (Katranitsas et al., 2003) and favour more tolerant exotic species (Piola and Johnston, 2006, 2008a). Urban stormwater drains increase chemical and metal pollution (Birch and Rochford, 2010a), which may influence natural colonization, as well as the effects of marine invaders

(Ruiz et al., 1999). Although, there has been significant investment to restore water quality in urban estuaries, we still have little information on the nature of the relationship between common urban pollution types and recovery of benthic communities (Díez et al., 2013; Pinedo et al., 2013).

In many estuaries, historical or anecdotal evidence suggests that healthy macroalgal assemblages were once more widespread but, in the absence of detailed historical data, we are not able to quantify these changes. Without adequate baseline records, manipulative studies using transplants represent an ecologically sensible way to identify causal mechanisms associated with environmental changes (Hernández-Carmona et al., 2000), and to guide recovery (Perkol-Finkel and Airoldi, 2010).

Restoration via the transplantation of healthy communities may be a viable solution if water quality assessments indicate improvements in environmental conditions (Campbell et al., 2014; Whitehead et al., 2010). Thus, a transplant experiment provides a test of whether stressors persist or conditions have improved sufficiently for re-establishment.

Our study was undertaken in one of the most heavily impacted estuaries in the world (Bloom and Ayling, 1977), the urbanized Derwent River estuary and the greater Derwent and adjacent D'Entrecasteaux Channel region. Historical concentrations of toxic metals in the vicinity of a zinc smelter and paper-pulp mill are among the highest ever encountered (Bloom and Ayling, 1977). In addition, considerable anecdotal and historical evidence indicates that the estuary has undergone massive ecosystem change (Edgar and Samson, 2004). Navigational charts suggest that kelp communities presented a shipping hazard in the nineteenth century (British surveys 1861 -1911) in locations where they are now absent (review in the National Archives of Australia). More recent impacts include marinas and a rapidly expanding aquaculture industry in the D'Entrecasteaux Channel (Oh et al., 2015).

A previous study examining the spatial distribution of sessile communities in the Derwent Estuary in relation to key pollution sources suggested that heavy metal contaminants and associated sediments are likely to have reduced occurrence of fleshy macroalgae, which have been replaced with filamentous algae and turf (Chapter 2). We used a replicated field experiment to further investigate these patterns, explore potential contemporary causal mechanisms and tease apart impacts of different pollution sources. This study also aimed to assess the potential for successful restoration of lost habitats within the estuary.

Specifically, we tested the response of natural rocky reef communities to four common anthropogenic stressors: marina pollution, sewerage, fish farm effluent, and stormwater discharge. These pollution types were chosen because they are common in urban estuaries and likely to increase in the future. To determine if 'natural' assemblages were able to survive at impacted and control locations, we transplanted mature assemblages grown in a nearby, and relatively unimpacted, location to sites in both the greater Derwent region and near the Hobart port and central business district. By transplanting mature healthy assemblages we were able to test whether: (1) the composition of healthy communities is differentially affected by different pollution types, (2) restoration potential varied between different sources/regions. We predicted that pollutants with nutrient enrichment could stimulate the growth of opportunistic species, given nitrogen deficiency in Tasmanian coastal waters (Wild-Allen et al. 2013), whereas metal/chemical contaminated impacts could result in decreased species diversity relative to controls. Therefore, we also tested whether functional groups on rocky reefs differed in response to impact types.

Materials and methods

Study areas

Our study region consisted of two connected marine embayments in the south-eastern coast of Tasmania, Australia (Fig 1); the Derwent Estuary, located adjacent to central Hobart and associated industry, and the D'Entrecasteaux Channel, adjacent to the predominantly residential greater Hobart region. The Derwent Estuary and the D'Entrecasteaux Channel are micro-tidal (0.8 m), with exposure to moderated oceanic swells, and wide entrances that promote efficient marine flushing (Whitehead et al., 2010). We chose these locations because they adjoin waterways with areas of potentially high macroalgal biodiversity and productivity (Scott, 2012), and similar urban development issues. Both are used for recreation, boating, fishing and marine transportation.

The Derwent Estuary is a drowned river valley that extends inland for 52 km with a total catchment area of 198 km^2 (Coughanowr, 1995). It is a salt-wedge estuary that is well mixed in the middle and lower regions, the regions containing our study sites. Average water depths are 10 - 20 m with a maximum depth of 44 m. The average flushing time is 15 days (Whitehead et al., 2010), which may help buffer against some impacts. Freshwater flows downstream along the eastern shore as a result of prevailing westerly winds (Butler, 2006). Hobart city's industrial zone is located adjacent to the middle estuary, and around 40% of Tasmania's population live around its shores (202 000 people; Coughanowr and Whitehead, 2013b). The adjacent D'Entrecasteaux Channel (446 km^2) is located between the mainland of Tasmania and Bruny Island, with a net flow of seawater from the D'Entrecasteaux Channel into the Derwent (Whitehead et al., 2010). Most of the waterway is > 10 m depth, reaching a maximum depth of 55 m. The estimated flushing time of the D'Entrecasteaux Channel waterway is 26 days (Herzfeld et al., 2008).

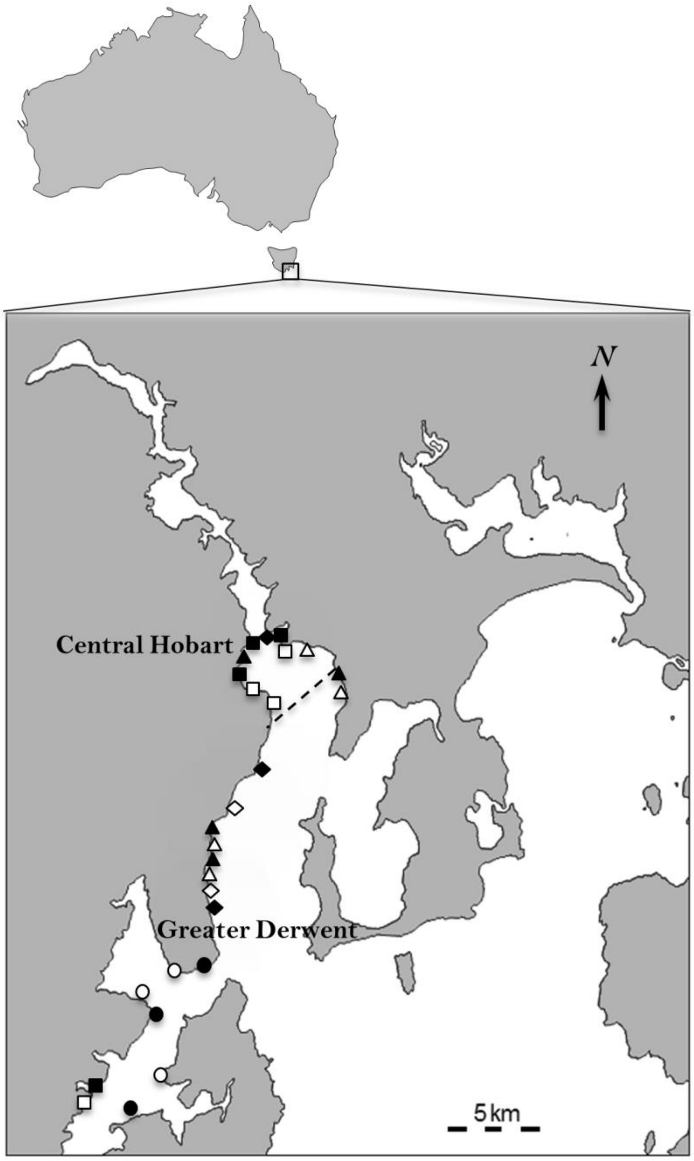

Figure 1. Location of the 27 sites used to test the effects of different urban impacts on the cover of the macro-algal and sessile invertebrate recruits in the Derwent and D'Entrecasteaux Channel (Greater Derwent), Tasmania Lat: -43.03 Long: 147.34. Solid symbols are the impact sites, open symbols are control sites. Diamonds = sewerage outfalls, squares = marinas, circles = fish farms, triangles = storm water drains. The dashed line shows sites within 5 km of the city centre.

Contemporary and historical contaminants

Nutrients, metals and organic contaminants enter the Derwent Estuary and D'Entrecasteaux Channel system from a variety of sources. Historical sources of contamination were industrial, including a zinc smelter (established in 1917) and paper mill (established in 1941) within the middle and upper estuary, respectively (Coughanowr and Whitehead, 2013b). Past industrial practices have resulted in heavy metal contamination of sediments and biota that is among the highest in the world (Whitehead et al., 2010). Despite reductions in metal outputs and a concomitant improvement of water quality, the majority of surface sediments still do not meet national sediment quality guidelines for arsenic, cadmium, copper, lead, mercury and zinc (Whitehead et al., 2010; ANZECC, 2000), with highly contaminated sediments occurring 40 km down the estuary. Contemporary sources of metal contaminants include urban stormwater run-off (Coughanowr and Whitehead, 2013b), antifouling paint fragments (Singh and Turner, 2009), and wastes associated with shipping operations, port facilities and marinas. This pollution remains an issue because metal contamination may be available to marine biota (Jones et al., 2013), especially after re-suspension.

Nutrients inputs are mostly contemporary and exhibit high spatial and temporal variability (Ross and Macleod, 2013), often making it difficult to partition nitrogen inputs between natural and anthropogenic sources. However, there are numerous salmon aquaculture operations in the D'Entrecasteaux Channel, with an estimated contribution of 1778 tonnes of fish waste, primarily as ammonia, into the system in 2009 (Whitehead et al., 2010), and with fish production increasing markedly since then. The other major point sources of nutrient pollution are sewage treatment plants that are distributed along the estuary. Due to the direction of flow and location of outfalls, elevated nutrient concentrations are largely retained within the system (Coughanowr and Whitehead, 2013b), constituting a persistent problem.

Experimental design and site selection

One hundred and thirty-eight cement garden pavers (300 x 300 x 50 mm), each ~8 kg in mass, were deployed at Tinderbox (43.0320°S, 147.3376°W), a central and comparatively unimpacted and well-flushed location within the system, at the head of the D'Entrecasteaux Channel (Fig 1.). Pavers were half covered with plastic (used later in a different experiment) and remained submerged at 3-4 m depth for 7 months, during which time a diverse community developed, which included mature fronds, representative of natural and 'healthy' assemblages in this region. Paver assemblages were dominated by canopy-forming algae such as *Ecklonia radiata, Carpoglossum confluens, Macrocystis pyrifera* and *Carpothamnion gunnianum,* with understorey species such as *Hemineura frondosa* and *Plocamium angustum* occurring in lower abundances.

Recipient sites were divided between 'Hobart central' (<5 km from the city port) and the 'greater Derwent region' (>5 km). Four types of pollution types were considered; marinas, stormwater discharge, sewage discharge and fish farms with four impacted sites and four associated control sites per pollution type. Pollution types were unavoidably non-randomly distributed through the estuary system. We commenced with 32 sites, however storms dislodged and overturned some pavers during the year, resulting in 27 sites at which data were collected at the end of the experiment.

Four pavers, and their associated mature algal assemblage, were randomly allocated and transplanted to sites. They were laid flat on rocky reef along the 2-4 m depth contour, approximately 0.5 to 1 m apart and at least 3 m from any naturally-occurring reef edge and sand scour. Marina sites were located <50 m from boat moorings inside marinas that harboured between 200-400 recreational and commercial vessels. Fish farm sites were located adjacent to marine farm leases in the greater Derwent region, <100 m from stocked cages. Leases typically had 4-8 cages (20-30 m in diameter), which were periodically moved within the lease. Pavers from one fish farm site were excluded from analyses as they were lost during a storm, as was a control site. Stormwater sites were located <20 m from the outlet of large concrete pipes (30-50 cm in diameter) that extended perpendicular to the shoreline. Sewage sites were located <10 m from the sewerage outlet. These distances and sites were chosen based on locations of nearby rocky reefs.

Pavers with mature algal assemblages were also deployed at control sites paired with each impact site, randomly selected from rocky reefs at least 1 km away from any potentially impacted site and other impact sources. This 1 km distance was assumed to be greater than that of direct impacts of the stressor (Di Franco et al., 2011; Oh et al., 2015). Pavers were transported in seawater-filled plastic bins, with transport time <1 hr.

Data Collection

The presence and relative abundance of macroalgal and sessile invertebrate species of paver communities was assessed when transplanted (time 0, the start of the experiment) and one year later (time 1). The transplanted community was evaluated by placing a 30 x 30 cm quadrat on each paver to delimit the sampling surface. String divided the quadrat into a grid, away from the paver edges; under each of 18 intersection points, the taxon was identified. Point counts of sessile organisms (algae and invertebrates) were manually recorded on an underwater slate. The majority of species were recorded to species level. Other taxa were recorded at the highest taxonomic resolution possible (e.g. red foliose algae). As a quality-control measure, a photograph was taken 0.5 metres above each block. The use of digital photography and collection of unknown species (not from the paver) allowed later identification of species unidentified in the field.

Statistical analysis

Multivariate analysis

Non-metric multidimensional scaling (MDS) and principal coordinate analysis (PCO) ordinations were examined to consider relative differences in community types between sites at time 0 and time 1. Multivariate analyses were conducted in PRIMER v6 with PERMANOVA (Primer-E Ltd, Plymouth, UK). Non-metric multidimensional scaling (MDS) was used to visualise the composition of sessile assemblages at the beginning and end of the experiment (pollution types and controls). We used Bray-Curtis similarity matrices and square root-transformed data as recommended for ecological data (Clarke and Warwick 2001) to reduce the contributions of dominant species. Initial assessment indicated that sites close to the city centre were quite different to elsewhere, so sites within this region were considered separate to sites outside of it in further analyses.

PERMANOVA was conducted to test the null hypothesis that community composition (at time 0 and time 1, separately) was not significantly different between pollution types and controls (Anderson et al., 2008). We included an index of wave exposure as a covariate, because wave exposure can affect macrophyte distribution and biodiversity (Nishihara and Terada 2010). Region was included in the model as a categorical term, as was a single interaction term, region x pollution type, to assess whether effects of the pollution types differed between the densely-populated middle estuary and sparsely-populated lower estuary. For this analysis, assemblage composition at time 0 and time 1 were considered separately because we were primarily interested in in the end result, of the new community composition under the pollution treatment result.

Type I sums of squares (SS) was used as the design, as we were using a covariate in the analysis (Anderson et. al 2008). Thus, the experimental design was: region, two levels (fixed); pollution sources and controls, five levels (fixed); location, 14 levels (random). Replicate pavers comprised the sampling unit. Location comprised a random factor that covered pairing of impact and control sites and accounted for the sub-regional variability along the estuary. Calculations of the Pseudo-F ratio and P value were based on 9999 permutations of the residuals under a reduced model.

To depict site differences in the community structure and to visualise correlations between pollution treatments and species in the two regions, principal co-ordinates analysis (PCO) was applied using the Bray Curtis similarity matrix (Anderson et al. 2008). The PCO was used to visualise patterns and correlations in unconstrained multivariate space using dissimilarity values, rather than ranks such as with MDS. Correlations with PCO axes were calculated using Spearman's correlation coefficients and displayed using vector diagrams on the PCO ordination.

Univariate analysis

Generalized linear mixed models were conducted to test responses to the different urban impact types on the cover of the different functional groups, using R statistical software version 3.1.0 (Core Team, 2014; R Core Team, 2013). Results relate to the whole estuary, but adjusted for region (near central Hobart or greater Derwent region) as a main effect. Wave exposure was determined using a fetch model, which uses the distance from a site to land in multiple directions as a proxy for potential wind-generated waves (Hill et al., 2010). Algal functional groups were defined on the basis of their structural dominance and hypothesised response to the pollution types. These functional groups were leathery canopy algae, foliose algae, corticated algae, and filamentous algae (Steneck and Dethier, 1994), which were tested for differences in groups in response to pollution types. In the generalized linear mixed models, a quasi-binomial distribution was assumed and a log link was employed for the cover data, the basic unit was at the paver replicate level. The model contained the fixed effects of pollution type (fish farms, marinas, sewerage outfalls and storm water drains) relative to the controls, region, wave exposure, and the random effect of location, as with the multivariate analysis.

Confidence intervals (CIs) and p-values were obtained for the model estimates for log response ratios, assuming a *t*-distribution with the appropriate degrees of freedom. The log response ratios for pollution type (and their CIs) were later converted to percentage change (or *n*-fold increase if it is greater than 100% increase) of the pollution type relative to their paired controls. The percentage change was calculated as *100*(exp(estimate)-1)* (and the n-fold increase as *exp(estimate)*). The CIs and p-values were used to assess significance of differences in the percentage cover of the different functional groups at the sites with the pollution types relative to their controls.

Results

Impacts on community composition

Macroalgal communities on pavers at time 0 were tightly clustered relative to time 1, one year later after transplantation (Fig. 2). PERMANOVA revealed that no statistically significant differences were evident at the start of the experiment between the composition of assemblages transplanted to the different pollution types (*Pseudo-F*=2.6, P > 0.06).

At the end of the experiment, a total of 60 algal taxa were identified from 27 sites. The nMDS ordination plot shows that after one year, assemblages on pavers at some sites were very different to the original community and quite dispersed in multivariate space (Fig. 2). Marina impact sites were farthest from the original community, whilst fish farm sites and some controls were relatively close.

Figure 2. Non-metric MDS ordination of time 0 (before transplantation) and four pollution types and controls after one year. Site-level averages of cover data were square root transformed and Bray-Curtis similarity used. Solid symbols indicate pollution impacts, clear symbols indicate controls. FF = fish farm, M = marina, S = sewage outlet; ST = stormwater outlet.

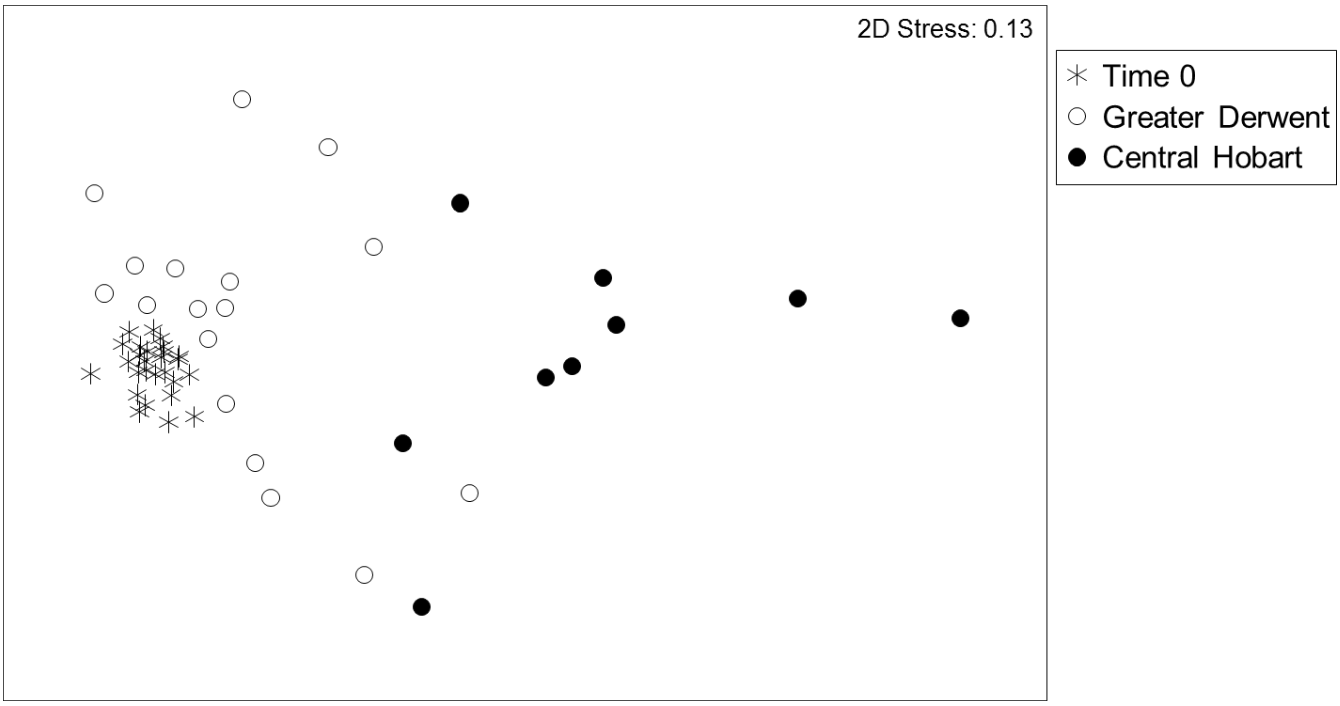

Figure 3. Non-metric MDS ordination of time 0 (before transplantation) and the regions after one year. Site-level averages of cover data were square root transformed and Bray-Curtis similarity used. Solid symbols indicate sites <5 km of the capital city; clear symbols indicate > 5 km.

Figure 3 depicts the transplanted communities across the estuarine region, and indicates a clear distinction and greater divergence of communities at sites near central Hobart at time 1 from those in the greater Derwent region and communities at time 0.

PERMANOVA analysis using multivariate data (Table 1) detected significant wave exposure effects. After one year, the structure of subtidal macrophyte assemblages also differed significantly between regions, pollution types and controls, and by location (Table 1). PERMANOVA also detected significant differences in the interaction between region and pollution type. The PCO plot based on pollution treatment effects indicates that filamentous algae, *Codium fragile* and *Ulva rigida* were associated with sites closer to marina impacts, while *Carpoglossum confluens* and most other species show the opposite trend (Fig. 4). The difference between marinas and other pollution types largely related to variation in cover of red filamentous algae, *Codium* spp., *Carpoglossum confluens* and the introduced species, *Aeodes nitidissima*. Figure 5 displays the strong region effects, with sites near central Hobart observed after one year showing a very different community to communities at the greater Derwent sites.

Table 1: Results from PERMANOVA based on Bray Curtis similarities of square root-transformed data from assemblages. P-values were obtained using 9999 permutations.

Source	Pollution Treatments			
	Df	MS	Pseudo-F	P(perm)
Exposure	1	17756	3.97	0.0003
Region	1	50671	9.17	0.0001
Pollution and controls	4	6845	2.32	0.0002
Location	12	4621	2.73	0.0001
Region x Pollution and controls	2	5942	3.51	0.0001
Res	79	1694		
Total	99			

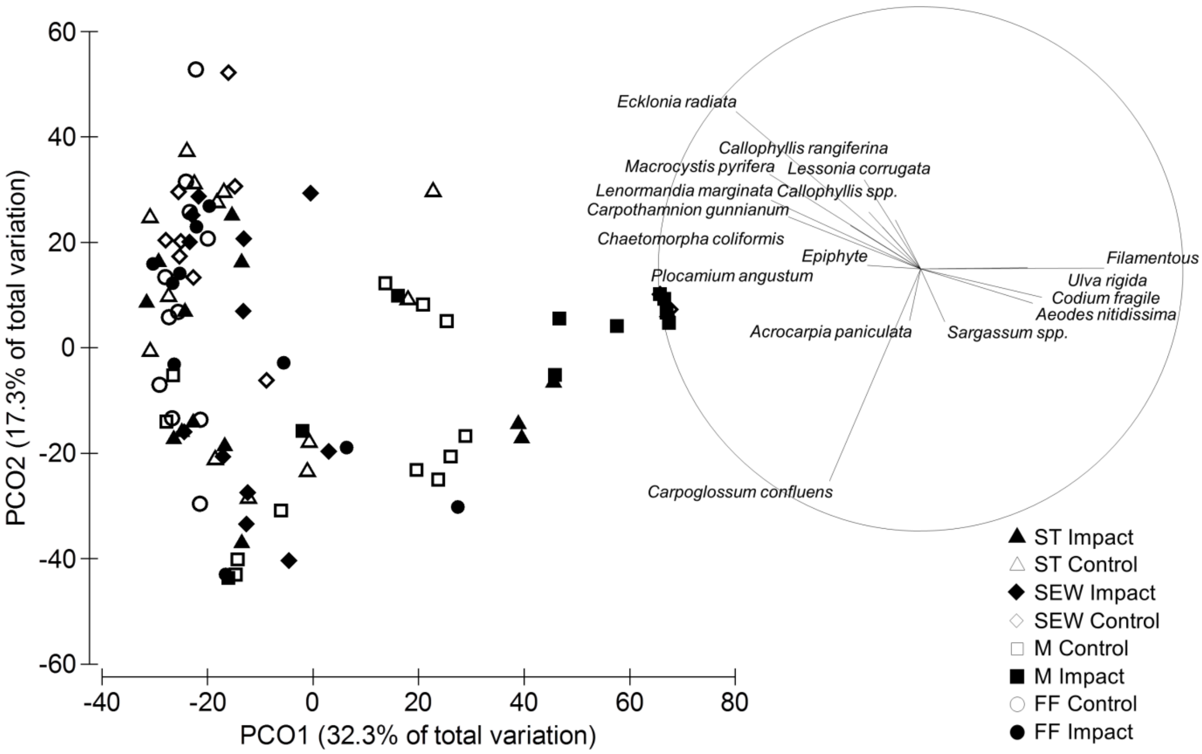

Figure 4. PCO ordination of paver community responses of the four different pollution types and the paired controls. Fitted species vectors for the taxa showing greatest correlation (Spearman's r > 0.3) with the first two axes are shown (circle indicates a radius of r=1). SEW = sewerage outfall, M= marinas, FF = fish farms and ST = storm water drains.

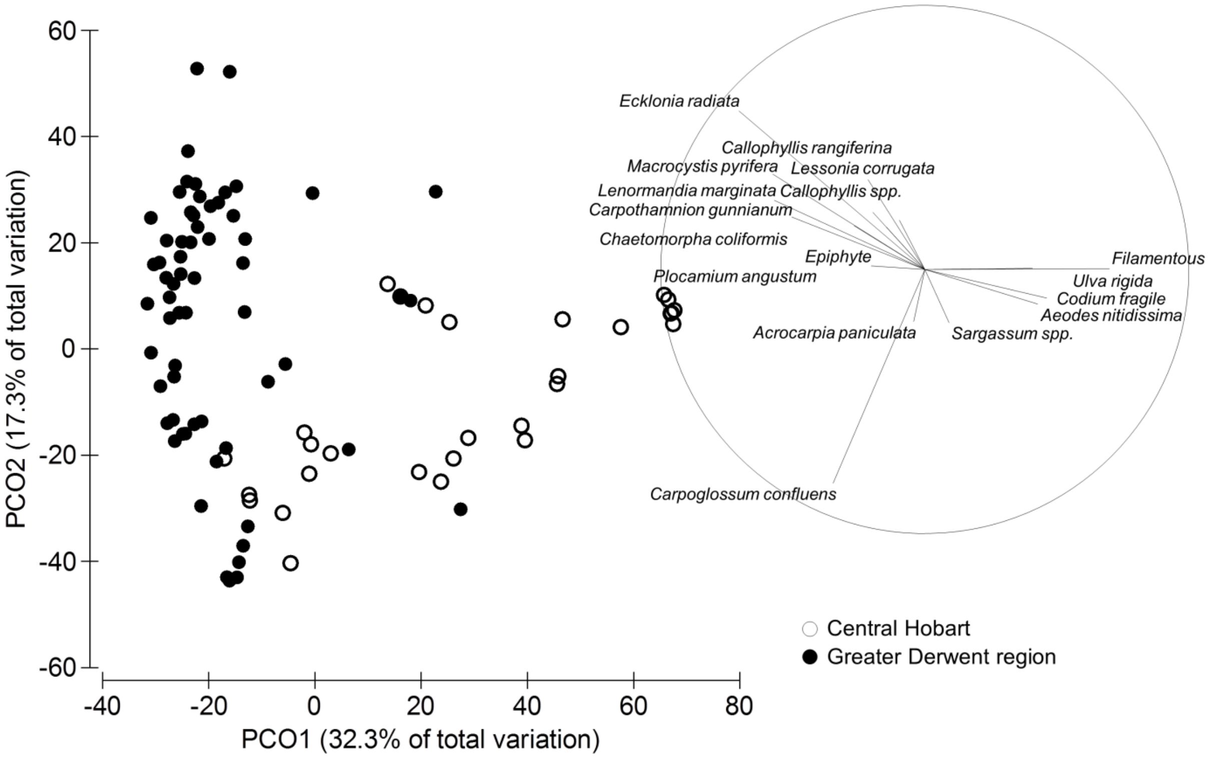

Figure. 5: PCO ordination of paver community responses of the four different pollution types and the controls by region. Fitted species vectors for the taxa showing greatest correlation (Spearman's r > 0.3) with the first two axis are shown (circle indicates a radius of r=1).

Impacts on algal functional groups

Univariate tests were conducted across the entire estuary gradient considering effects of wave exposure, region and location. The log response ratio analyses revealed significant variability in algal functional groups among pollution impacts (Fig. 6). Fish farms had a strong positive effect on cover of filamentous algae, with a 53-fold increase at impacted sites ($t = 3.88$, $df = 79$, $P < 0.01$), whereas canopy, foliose and corticated algae were not significantly affected (Fig. 6). Marinas significantly depressed canopy algae (-43% change, $t = -2.33$, $df = 79$, $p = 0.02$) and foliose algae (-90% change, $t = -3.11$, $df = 79$, $p < 0.01$) while the cover of filamentous algae increased 10-fold ($t = 4.96$, $df = 79$, $P < 0.01$). Filamentous algal cover also increased 9-fold adjacent to stormwater drains relative to the controls ($t = 4.46$, $df = 79$, P = $P < 0.01$). No algal functional groups were significantly affected by sewerage, and stormwater inputs did not affect canopy, foliose or corticated algae (Fig. 6).

Regional and covariate effects on macro algal assemblages were evident. A significant 70% loss in canopy algae was observed in the Hobart region, ($t = -6.56$, $df = 12$, P = $P < 0.001$), whilst foliose ($t = 2.61$, $df = 12$, $P < 0.05$) and filamentous algal cover experienced a 47-fold increase (t = 4.6, df = 12, P < 0.001). As expected, low wave exposure decreased canopy cover (t = -2.93, df = 79, P < 0.01) and increased filamentous cover (t = 4.28, df = 12, P < 0.001).

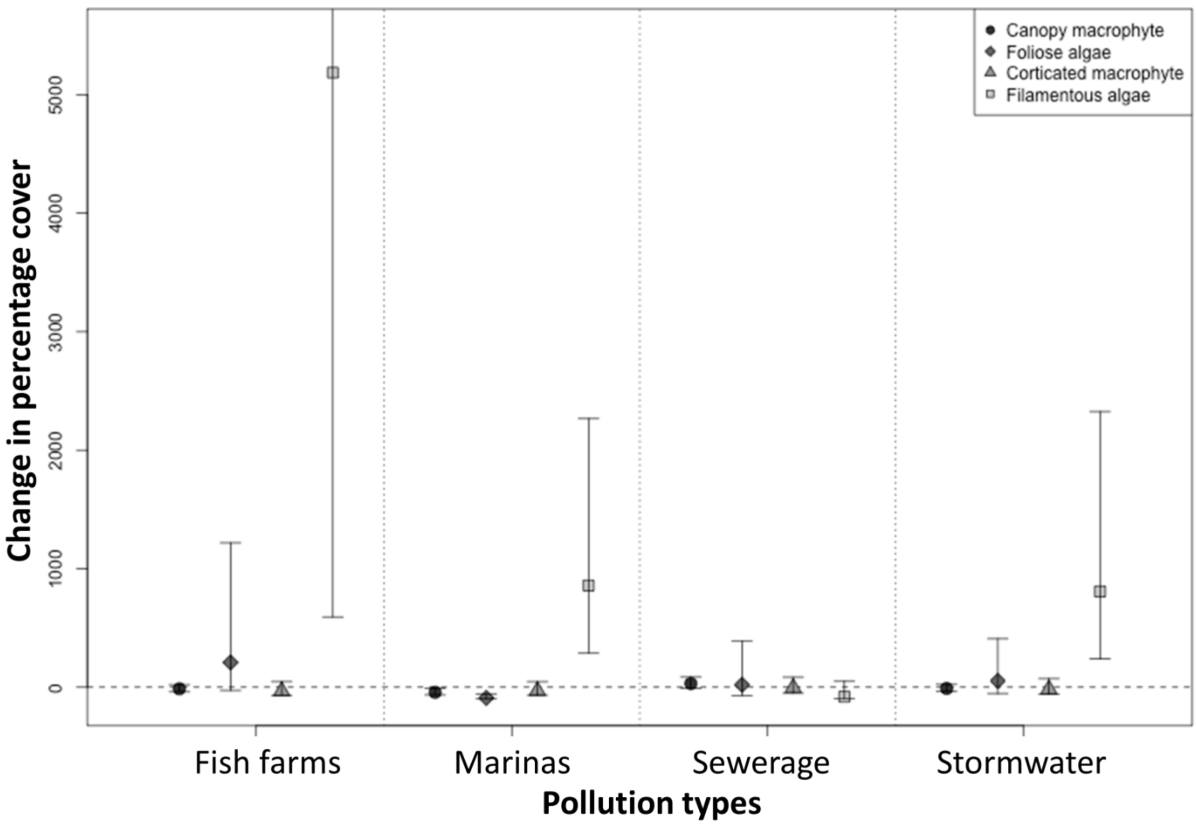

Figure 6. Variation in algal cover for polluted sites relative to controls for different functional groups of transplanted macroalgae, after effects of wave exposure, region and location are considered. Means and 95% confidence intervals are derived from linear mixed models of log response ratio against pollution types.

Discussion

To our knowledge, this is the first study investigating the responses of transplanted sessile communities on subtidal rocky reef to multiple urban pollution sources in a location where canopy forming species were once abundant but have disappeared. Our results supported the hypothesis that after 1 year's exposure, transplanted 'healthy' algal communities were differentially affected by the different pollution types tested. The multivariate analysis indicated strong region and pollution type effects. Sites near central Hobart had a different community compared to the greater Derwent region, presumably because they are subject to a greater range and/or intensity of urban impacts, including unmeasured factors. This important pattern was considered in univariate tests in which marinas showed the strongest negative influence, through major decreases in canopy and foliose macroalgal cover. Filamentous algae were the most consistent indicator of pollution, increasing significantly near fish farms, marinas and storm water drains.

Community response to pollution types

Quantifying the response of rocky reef communities to pollution impacts, and separating these from underlying natural variability within a complex estuarine ecosystem over a long time period in the field, is exceptionally difficult (Veríssimo et al., 2013). Transplanted assemblages were similar for each pollution type at the beginning, but after one year, communities subjected to the pollution treatments differed significantly, even though there was some unavoidable spatial clumping near Hobart region. The strong effects between regions were accounted for in the univariate analyses. We speculate that reefs near central Hobart were impacted by multiple sources of urban pollution, historical and contemporary sources. After one year, structurally-diverse communities transplanted to within 5 km of the city were largely lost, replaced by filamentous and foliose algae. Given the legacy of pollution in the city region, control sites were almost certainly also contaminated by broad-scale impacts of nutrients and heavy metals, regardless of the fact that these sites were located >1 km from the point sources of pollution that provided the focus of this investigation.

In our study, environmental effects associated with proximity to city (upper region) reduced canopy macrophytes. This difference, for robust wave-resistant algae, may be linked to the exposure gradient (Hill et al., 2010), although anecdotal evidence and old nautical charts suggest that laminarian kelp was once diverse and abundant at sites near central Hobart. The loss of canopy algae is more likely related to the direct cumulative effects of urban stressors, particularly nitrogen loading and historical heavy metal contamination (Coughanowr, 1995). Kelp transplanted to impacted and control sites near central Hobart did not survive, consistent with a global trend for declining canopy-forming algae near urban coasts (Airoldi and Beck, 2007; Connell et al., 2008; Díez et al., 2014; Steneck et al., 2002). Kelps often dominate reef assemblages and have a strong influence on the physical and biological environment through provision of habitat, water baffling and shade (Edgar et al., 2004; Wernberg et al., 2003). In addition, foliose algae (e.g. *Ulva rigida* and *Aeodes nitidissima*) increased near central Hobart compared to the greater Derwent region, as did filamentous algae. These two groups are both known to prefer low wave exposure areas and to be indicators of nutrient enrichment (Arévalo et al., 2007; Diez et al., 1999).

Our data are consistent with prior studies indicating that accumulated urban impacts can lead to species extinctions, severe seascape changes, and homogenization of biota (Airoldi et al., 2015). Reef communities near central Hobart have historically been affected by heavy metal contamination, which persists at high levels (Whitehead et al., 2010), and also by high nutrient levels due to the majority of sewerage outfalls being located in the middle estuarine reaches (Coughanowr, 1995; Wild-Allen et al., 2013). The Greater Derwent region has not been subjected to the same scale of cumulative and continuing impacts, and communities in these locations were less impacted near pollution point sources.

The composition of transplanted communities near central Hobart demonstrates that the estuary has now changed to the extent that the system is unlikely to naturally return to a structurally-complex community in the short- to medium-term. This outcome is not surprising given the prevalence of multiple stressors (Folke et al. 2004; Strain et al., 2014), including contamination by antifoulants (e.g. Stupak et al., 2003), metals (Bloom and Ayling, 1977), and chemicals (Di Franco et al., 2011; Thibaut et al., 2005), and high water turbidity (Torres et al., 2008, Lewis et al., 2009, Gremare et al 1998). High concentrations of metals are often present in industrial waters (Xia et al. 2012), and the Derwent is one of the most impacted estuaries in the world (Bloom and Ayling, 1977).

Although, pollutant-related declines of Fucales have been reported previously (Diez et al., 1999; Sales et al., 2011), including in the Mediterranean where several taxa are now regionally extinct (Thibaut et al., 2005), *Carpoglossum confluens* was more tolerant than *Ecklonia radiata* and survived at control locations near central Hobart. This Tasmanian situation is similar to that in Sydney, one thousand kilometres north, where the transplanted fucoid *Phyllospora comosa* has survived in urban settings (Campbell et al., 2014). In the Derwent, transplants of *C. confluens* could usefully assist ecosystem restoration.

Responses of different functional groups to pollution types

Fish farms, marinas, and stormwater sites were consistently characterised by a dominance of filamentous algae, while algae did not respond consistently to sewage inputs here. Our results support previous studies suggesting that communities in heavily impacted locations are dominated by tolerant species with morphological and physiological adaptations (Peng et al., 2013), smaller growth forms (Airoldi et al., 1998, 2008), and simpler and shorter life histories (Stuart-Smith et al., 2015).

In the same region, Oh et al. (2015) found substantial overgrowth of natural reefs by filamentous algae up to 400 m distance from fish farms, probably due to release of nutrients. Nutrient enrichment (via fish farms and sewage outfalls) is known to promote the dominance of competitively superior and rapidly-growing species (Hillebrand et al., 2007), such as *Chaetomorpha coliformis* and *Ulva rigida*. Opportunistic species generally perform better than others in these situations, growing quickly and thereby becoming competitively dominant (Vinueza et al., 2014). In addition, nutrients are likely to accumulate in sheltered waters with low flushing (Russell and Connell, 2007), thus these areas are more at risk from enrichment.

Marinas significantly increased filamentous cover but also depressed canopy and foliose algae. These results agree with those of another study examining marina influences, on

Mediterranean rocky shores (Benedetti-Cecchi and Osio, 2007), but contrast with a study assessing effect of marinas on shallow subtidal assemblage structure in Italy, where little effect of marinas was found (Di Franco et al., 2011). Clearly, harbours and marinas are complex coastal habitats with many sources of anthropogenic disturbance (Lenihan et al., 1990), including accumulation of numerous inorganic chemicals (Callier et al., 2009; Johnston et al., 2011). Prior studies consistently highlight the harmful effects of metals (Correa et al., 1999), antifouling paints (Johnston and Keough, 2005), and toxic chemicals (Bocchetti et al., 2008), all often found near marinas.

Our study revealed a clear increase in filamentous algae near stormwater drains. Although not surprising, this result differs from previous studies, which have experienced difficulties in assessing stormwater impacts due to the unpredictable and episodic nature of release events (Beck, 1996; Roberts et al., 2007), sometimes not finding any impact on macroalgal assemblages (Cox et al., 2013). An investigation in Hawaii, however, also found storm-drain effluent to favour the growth of opportunist algal species, although in that case these were non-indigenous species (Lapointe and Bedford, 2011).

Opportunistic species have generally been found to increase near sewerage outlets (Arévalo et al., 2007). Surprisingly, we did not find this result, perhaps because of high spatial variability in this system or because nutrients are sufficiently dispersed from sewerage outlets near Hobart to inhibit the detection of local effects (Coughanowr, 1995).

Conclusion

The Derwent estuarine ecosystem has been negatively affected by extensive urbanisation near central Hobart. Loss of complex algal communities in this region represents a major change in community structure that is probably difficult to remediate (Folke et al. 2004). Nevertheless, changes in reef assemblages can potentially be partly reversed in lesser impacted areas through translocation of resistant perennial macro-algae, in this case the fucalean alga *Carpoglossum confluens* rather than laminarian taxa.

Over the broader region, marinas appear to have exerted a greater influence on reef communities than other pollutant sources examined, with larger perennial algae replaced by filamentous algae and other species with lesser structural complexity. Loss of healthy algal communities presumably has additional flow on impacts on fish and invertebrate populations (Schiel and Foster, 2006; Stuart-Smith et al., 2015b).

Management strategies for urban contaminants need to recognise the ubiquity of cumulative impacts. Filamentous algae provide a simple indicator of fish farm, marina and stormwater influences, but interpretation of this indicator additionally depends on knowledge of pollution loadings and the natural state of the ecosystem. Overall, the magnitude of change in the Derwent and other urbanised estuaries has apparently been huge, warranting more attention and action to improve protection for these critical marine habitats.

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Chapter 4. Effects of different urban impacts on the recruitment of sessile estuarine reef biota

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Abstract

Effects of four different urban pollution sources on the recruitment and development of macro-algae and sessile invertebrates on rocky reefs were assessed in a degraded south-eastern Australian estuary. Establishment of sessile communities to bare substrate provided by experimental pavers over the course of one year was strongly and differentially influenced by nearby marinas, storm-water drains, sewerage outfalls and fish farms. Marinas, in particular, dramatically reduced recruitment of both the number and cover of native species relative to control sites. Non-indigenous and cryptogenic recruits increased on experimental pavers placed near marinas and sewerage outlets. Fast-growing opportunistic cover was significantly higher near fish farms and sewage outfalls, with cover of native species amplified at the latter relative to control sites. The extent of pollution impacts on native biodiversity warrants wider recognition of the issue of degraded estuarine condition, and increased management intervention.

Key words: Recruits, urban impacts, algae, sessile invertebrates

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Effects of different urban impacts infrastructures on estuarine reef biota

Introduction

Many of the world's major urban centres have developed on large estuaries, often exploiting the land–sea interface (Waltham and Connolly, 2011). Continuing population growth in these areas has resulted in a proliferation of marinas, sewerage outfalls, stormwater drains, fish farms, and associated discharged contaminants (Airoldi et al., 2015; Dafforn et al., 2015). More than 50% of the coastline has been modified in some parts of Japan (Koike, 1996), Australia (Chapman and Bulleri, 2003), USA (Davis et al., 2002) and Europe (Airoldi et al., 2005). These urban impacts vary in extent and intensity by altering flow rates and delivering pollutants, which places pressure on the adjacent near-shore rocky reef biota (Airoldi and Bulleri, 2011; Strain et al., 2015; Vitousek et al., 2007). The effects of multiple anthropogenic stressors are often amplified in estuaries, which are the most polluted of all marine ecosystems (Edgar et al., 2000; Roy et al., 2001). Interactions between urban impacts and other stressors (Crain et al., 2008; Strain et al., 2014; Wahl et al., 2011) can also have widespread impacts on the recruitment and survival of reef biota at local scales (Wernberg and Connell, 2008) with flow-on effects on numerous other rare (Schmidt and Scheibling, 2007), endemic (Dayton et al., 1998; Scott, 2012) and commercially-valuable (Bertocci et al., 2015) species.

Recruitment of perennial canopy forming algae and sessile invertebrates on rocky reefs is a key process in temperate marine systems (Airoldi and Beck, 2007; Gorman and Connell, 2009; Gorman et al., 2009; Mangialajo et al., 2008). Recruits are strongly influenced by physical conditions, competition and local community structure and species richness (Schiel & Foster 2006; Bulleri 2009). Some invertebrates have pelagic larval stages that can disperse widely (Keough 1998), but dispersal of some macroalgal propagules are highly localized, down to only 1 m (Kendrick and Walker 1995). Other sessile marine species in shallow subtidal reef habitats (e.g. ascidians) produce short-lived propagules (minutes to hours), thus recruitment can vary at small spatial scales (Sams and Keough 2012) and high variability among sites. Pollution events can potentially disrupt densities and survival of the early life stages of marine organisms (Vadas et al., 1992) and have the potential to be an important structuring force on benthic assemblages that form the habitats for many marine species (Johnston et al., 2002). Many studies on the impacts of urban pollution have focused on single species and adult responses, where pollution often results in the rapid or gradual loss of species from urbanised environments from pulse stressors (Díez et al., 2014). However, comparatively little is known about community level effects on early settlement and survival of the sessile community. It is equally possible that pollutants may inhibit the recruitment of species back to impacted locations, causing a bottleneck in succession and preventing recovery of natural habitats.

Thus, urban pollution may inhibit the settlement and establishment of sessile biota. Nutrient enrichment can negatively affect fertilisation of native algae and increase mortality of germlings (Coelho et al., 2000; Kevekordes and Clayton, 2000). Settlement of suspended sediments can smoother small recruits close to the substrate surface (Strain et al. 2015), promoting the growth of opportunistic species with quick turnover rates (Eriksson et al., 2002). Ports and marinas are rich in heavy metals, and can cause selective mortality of the early life stages of native algae (Coelho et al., 2000) and sessile invertebrates (Johnston and Roberts, 2009; Medina et al., 2005; Schiff et al., 2004), and reduce the species richness of native communities (Dafforn et al., 2008).

Documented losses of native sessile communities in estuaries (Dafforn et al., 2012; Johnston et al., 2011 and in previous chapter), represent a form of disturbance regime that will result in open substrate and successional opportunities. Succession theory and the history of invasive species impacts (Ruiz et al., 2000, Johnston et al., 2011, Piola and Johnston et al., Levine et al., 2000) suggest that invasive and rapidly-growing opportunistic species may capitalise on empty niches (Stuart-Smith et al., 2015). In addition, the construction of coastal infrastructure can support elevated abundance of exotic species (Dafforn et al., 2009) and may act as ‘corridors’ for the spread of undesirable species into natural communities (Airoldi et al., 2015). Yet relatively little is known about any differential impacts of urban pollution sources on the colonization of sessile biota and early stages of succession on areas of hard substrate in temperate estuaries (but see Dafforn 2008).
In the present paper, we assess the effects of different urban impacts on functional groups, richness and cover of native and non-indigenous algae and sessile invertebrates that recruit on experimental pavers placed on rocky reefs in one of Australia’s most impacted urban estuaries (Bloom and Ayling, 1977). We use a replicated experimental design to test for different sessile biota recruiting to reef sites near marinas, storm water drains, sewerage

outfalls and fish farms. We hypothesized that sites near marinas and storm water drains, where inorganic and potentially toxic pollutants enter the system, would possess an early successional community comprising lower richness and cover of sessile biota relative to control sites (Jonston and Roberts 2009) and those at fish farms and sewage drains, where organic pollution should result in higher cover of opportunistic species, which would dominate and exclude slower growing non-opportunistic species (Duarte 1995, Littler and Littler 1980). We also investigated patterns in invasive species, and hypothesised greater recruitment of non-native species would occur on experimental pavers places near marinas, given anthropogenic structures such as marinas and jetties have been found to harbour disproportionate densities of non-native sessile species in other cities (Airoldi et al. 2015, Piola and Johnston 2008).

Materials and methods

Study Sites

Recruitment pavers were placed at 27 sites along the lower Derwent estuary and the D'Entrecasteaux Channel, SE Tasmania, Australia (Fig 1), as described in an associated study of survival of transplanted algal communities (Chapter 3). Although the Derwent Estuary extends inland for 52 km, the studied region in the lower estuary is largely dominated by marine water. The D'Entrecasteaux Channel also contains marine water which flows northward into the Derwent estuary (Whitehead et al., 2010).

The Derwent estuary and D'Entrecasteaux Channel region (hereafter referred to as the study region) comprise a model system because they are representative of impacts experienced in many urban estuaries. As with almost every other inshore region of the world, major ecosystems changes have occurred due to anthropogenic impacts over the last two centuries (Crawford et al., 2000; Edgar and Barrett, 2000). The study area has a long history of extensive foreshore development, including establishment of commercial and recreational boating marinas from 1802, storm water drains and sewerage outfalls not long after, and more recently the introduction of salmon farming in the 1980s in the D'Entrecasteaux Channel.

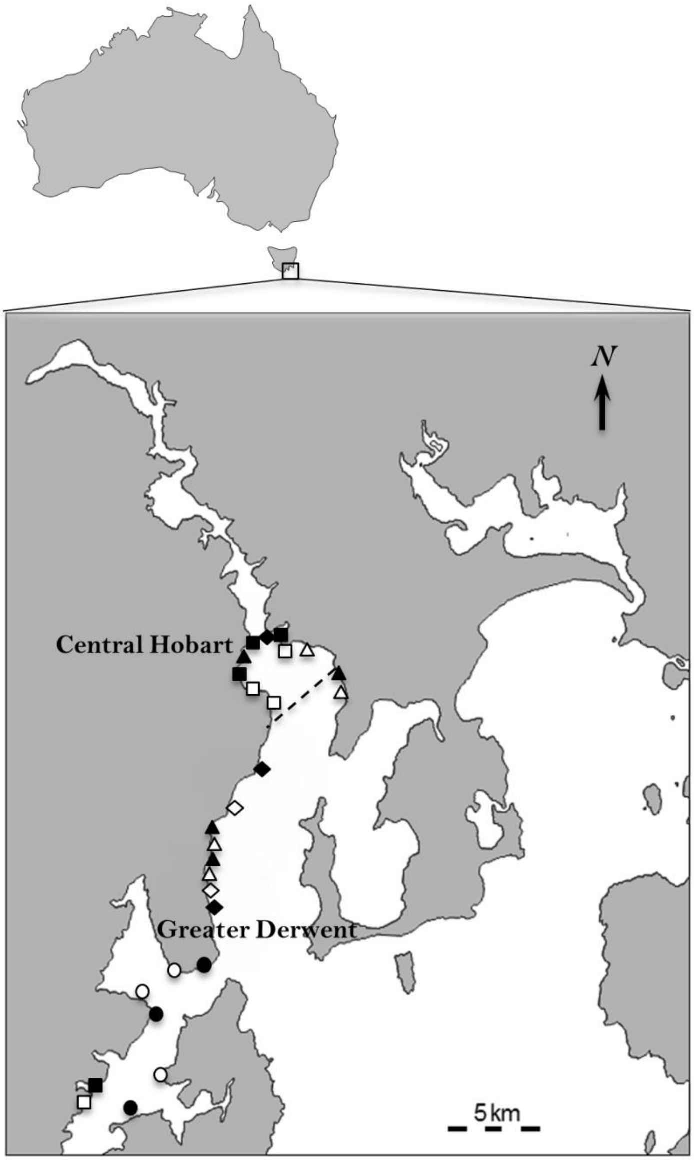

Figure 1. Location of the 27 sites used to test the effects of different urban impacts on recruitment of sessile communities in the Derwent and D'Entrecasteaux Channel, Tasmania Lat: -43.03 Long: 147.34. Dashed line indicates sites within 5 km of the central business district of Hobart. Solid symbols are the impact sites, open symbols are control sites. Diamonds = sewerage outfalls, squares = marinas, circles = fish farms, triangles = storm water drains.

Urban impacts on algae and sessile invertebrate recruits

A total of one hundred and twenty eight 0.3 × 0.3 m pavers were deployed for one year (March 2011-2012) across the study region. Concrete pavers presented a rough surface to facilitate recruitment by algal spores and sessile invertebrates (Park et al., 2014) and supported growth of dense native macroalgal communities in a previous experiment (Ch 3). Four pavers were placed at each of the four sites, each in close proximity to four different urban impact types (fish farm, marinas, sewerage outfalls and storm water drains). Four pavers were also placed in nearby rocky reefs at sites ~1 km from the impacted sites, as controls (Fig. 1). Pavers were deployed on rocky reef along the 2-4 m depth contour, approximately 0.5 to 1.0 m apart and at least 3 m from the naturally-occurring reef-sand interface. The marina sites were located <50 m from permanent boat moorings in marinas that harboured between 200-400 recreational and commercial vessels. Fish farm sites were all located in the D'Entrecasteaux Channel <100 m from stocked cages. Storm water pipe sites were located <20 m from the pipe outlet, which consisted of a large concrete pipe (30-50 cm in diameter) that extended into the estuary perpendicular to the shoreline. Sewerage sites were located <20 m from the pipe outlet.

At the end of the experiment (March 2012) a 0.3 × 0.3 m quadrat was placed on each paver. The percentage cover of different sessile taxa was estimated by counting the number of times each taxon occurred directly under 18 string intersection points, dividing by the number of points counted, and multiplying by 100. Taxa were identified to species level where possible, or aggregated at higher taxonomic levels otherwise. Abiotic variables (sediment and bare paver surface) were not included in the assessment of the community or univariate percentage cover, but were considered separately as a measure of potential recruitment space. During the experiment, 30 pavers were lost or turned over by storms. These missing replicates were located at marina control sites (4 replicates), sewerage outfall sites (4 replicates), sewerage control sites (8 replicates), stormwater drain sites (2 replicates), stormwater control sites (3 replicates), fish farm impact sites (4 replicates), and fish farm control sites (5 replicates). The remaining pavers were taken back to the laboratory at the end of the experiment, allowing species identities to be checked where necessary using microscopes.

Multivariate analyses

The effect of the different urban impact types on the benthic community assemblage was analysed using canonical analysis of principal coordinates (CAP) (Anderson, 2003). CAP was "constrained" by pollution type to maximise separation between the impact types and control sites. To reduce variability in outputs, data were averaged at the site level. The resultant 'site × species' matrix was square root transformed and converted to a Bray Curtis similarity matrix. We used a Spearman correlation (R) between the canonical axes and density of taxa to measure the contribution of individual taxa on the differences between the control and anthropogenic impact sites. These correlations are shown visually as a vector plot for taxa with $R > 0.5$. All multivariate analyses and plots were conducted with the package PRIMER 6.1.13 (Anderson et al., 2008; Clarke, 1993).

Univariate analysis

Taxa identified during this study were classified into broad functional groups: abiotic or biotic; nonindigenous/cryptogenic or native; opportunistic or non-opportunistic species (Table 1). Opportunistic species were defined as fast growing, ephemeral, and with continuous periods of reproduction (Lobban and Harrison, 1994). Non-opportunistic algae and invertebrates were slower growing, with a long life span, perennial, and with shorter periods of reproduction (Littler and Littler, 1980). In addition, we classified macroalgae into four functional categories defined by Steneck and Dethier (1994): canopy-forming, foliose, corticated and filamentous algae.

The effects of the different urban impact types on the number of species and the cover of the different functional groups were tested using generalized linear mixed models. A quasi-poisson distribution was assumed for the number of species and a quasi-binomial distribution was assumed for the cover data. A log link was employed for both data types with individual paver as the basic replicate unit. The model included the fixed effects of urban impacts (fish farms, marinas, sewerage outfalls and storm water drains) relative to the controls, region, wave exposure, and the random effect of site. These random effects were included in the model to adjust for the natural variability between sites. Region was a categorical variable that distinguished sites within 5 km of the capital city, Hobart, from the greater Derwent region (>5 km), as sites near the capital city have been identified to be generally more heavily impacted (Ch 3). Exposure was measured using a fetch model, which uses the distance of 14 vectors from a site to any mass of land in multiple directions as a proxy for potential wind-generated waves (Hill et al., 2010).

All univariate analyses were carried out with the R statistical software version 3.1.0 (Core Team, 2014; R Core Team, 2013). Confidence intervals (CIs) and p-values were obtained for the model estimates (or log response ratios), assuming that they are *T*-distributed with the appropriate degrees of freedom. The log response ratios for the urban impacts (and their CIs) were further converted to percentage change (or *n*-fold increase if it is greater than 100% increase) of the urban impact relative to the controls. The percentage change was calculated as *100*(exp(estimate)-1)* (and the n-fold increase as *exp(estimate)*). The CIs and p-values were used to assess significance of differences in the number of species or percentage cover of the different functional groups at the sites with the urban impacts relative to their controls.

Results

Effect of different urban impacts on benthic community composition

During the experiment, fifty-one species of algae (94%) and three species of (6%) sessile invertebrates recruited to the pavers (Table 1). In general, algae and sessile invertebrates had low cover on the pavers. The most abundant species were *Ulva rigida, Polysiphonia* sp., brown algal turf, *Aeodes nitidissima* and *Codium fragile*.

There were minor differences between impacts (fish farms, sewerage outfalls and storm water drains) and strong differences for marinas, and their paired control sites along the first two axes of canonical space (Fig. 2). The greatest differences in sessile community structure were observed between the marina impact and control sites (*Codium* spp. R = 0.52; *Grateloupia* sp. R = -0.60; Fig. 2). Some differences were also evident between the fish farm and sewerage pipe impact sites and control sites (*Chaetomorpha coliformis* R = 0.59, *Polysiphonia* sp. R = -0.51; Fig. 2). In contrast, little difference was evident between the stormwater drain impact and control sites (Fig. 2).

Table 1: Summary of taxa found during the experiment and the source of their classification as native (N) or non-indigenous (NIS), cryptogenic (C), opportunistic (O) or non-opportunistic (NO).

Species name	Status	Life history	Source
Acrocarpia paniculata	N	NO	Womersley (1987)
Aeodes nitidissima	NIS	OP	Scott (2012)
Ballia callitricha	N	NO	Womersely (1998)
Barnacles	N	OP	Aquenal Pty Ltd (2002)
Branched brown algae	N	NO	G. Kraft pers. comm.
Brown filamentous algae	N	OP	Aquenal Pty Ltd (2002)
Bryozoans	N	OP	included all species
Callophycus harveyanus	N	NO	Womersley (1994)
Callophycus spp.	N	NO	Womersley (1994)
Callophyllis rangiferina	N	NO	Womersley (1994)
Callophyllis spp.	N	NO	Womersley (1994)
Carpoglossum confluens	N	NO	Womersley (1987)
Carpothamnion gunnianum	N	NO	Womersely (1998)
Caulerpa longifolia	N	NO	N. Barret pers. comm.
Caulerpa trifaria	N	OP	N. Barret pers. comm.
Caulocystis cephalornithos	N	NO	Womersley (1987)
Chaetomorpha coliformis	N	OP	Valiela *et al.,* (1997)
Champia viridis	N	NO	Womersley (1996)
Cladostephus spongiosus	N	NO	Womersley (1987)
Codium fragile	NIS	OP	McDonald et al., (2015), Aquenal Pty Ltd (2002)
Codium sp.	NIS	OP	Womersley (1984), Aquenal Pty Ltd (2002)
Crassostrea gigas	NIS	OP	Ruesinslow *et al.* (2005)
Crustose coralline algae	N	NO	Stenecslow *et al.* (1986)
Curdiea spp.	N	NO	Womersley (1996)
Cystophora sp.	N	NO	Womersley (1987)
Delisea spp.	N	NO	Womersley (1996)
Dictyota dichotoma	C	OP	Aquenal Pty Ltd (2002)
Ecklonia radiata	N	NO	Womersley (1987)
Foliose red algae	N	OP	A. Fowles pers. obs.
Glaphyrymenia spp.	N	NO	Womersley (1994)
Gracilaria secundata	N	OP	Womersley (1994)
Grateloupia spp.	C	OP	Womersley (1994)
Green filamentous	N	OP	Oh *et.al* (2015)
Griffithsia spp.	N	OP	Womersely (1998)
Haliptilon roseum	N	NO	Womersley (1996)
Halopteris spp.	N	NO	Womersley (1987)
Hemineura frondosa	N	NO	Womersley (2003)
Hincksia sordida	N	OP	Womersley (1987), Kraft (2009)
Laurencia spp.	N	OP	Womersley (2003)
Lenormandia marginata	N	NO	Womersley (2003)
Lessonia corrugata	N	NO	Womersley (1987)
Macrocystis pyrifera	N	NO	Womersley (1987)
Myriogramme gunniana	N	NO	N. Barret pers. comm.
Perithalia caudate	N	NO	Womersley (1987)
Plocamium angustum	N	NO	Womersley (1994)
Polysiphonia sp.	C	OP	Womersley (1981)
Red filamentous algae	C	OP	Aquenal Pty Ltd (2002)
Red foliose epiphyte	N	OP	Aquenal Pty Ltd (2002)
Rhodymenia australis	N	NO	Womersley (1996)
Sargassum spp.	N	NO	Womersley (1987)
Sporochnus sp.	N	OP	Womersley (1987)
Turf brown algae	N	OP	G. Kraft pers. comm.
Ulva rigida	N	OP	Lavery and McComb (1991)
Undaria pinnatifida	NIS	OP	Valentine (2007)

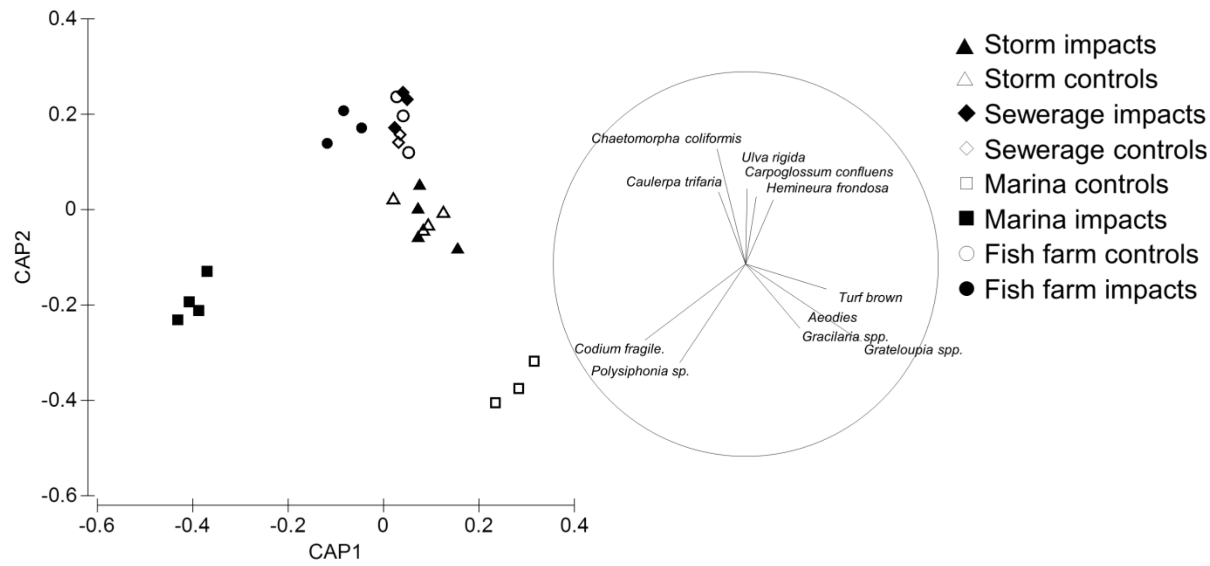

Figure 2: Canonical analysis of principal coordinates (CAP) ordination showing differences in the sessile community recruited to pavers near urban contaminant sources (solid symbols) and their paired control sites (open symbols). Taxa that contributed to the greatest dissimilarity between the impact and controls are shown by vector plot, with circle diameter showing correlation of 1.

Effect of different urban impacts on species richness and percentage cover of different functional groups

A significantly greater number of cryptogenic and non-indigenous species (e.g. *Crassostrea gigas*, *Polysiphonia* spp. and *Codium fragile*) were found near marinas (2.7- fold increase, $t = 2.9$, $df = 79$, $p < 0.01$) and sewerage pipe outlets (6.1- fold increase, $t = 2.11$, $df = 79$, $p = 0.04$) than at associated control sites (Fig. 3a), although there was high variation between sites. In contrast, no significant differences were found between the stormwater effluent (78% increase, $t = 1.61$, $df = 79$, $p > 0.05$) or fish farms (25% increase, $t = 0.26$, $df = 79$, $p > 0.05$), and their paired controls (Fig. 3a). The number of native species at the marina sites was significantly lower than at paired controls (- 96% change, $t = -3.45$, $df = 80$, $p < 0.001$), whilst pavers associated with other urban impact types showed no detectable changes in the number of native species compared with their control sites (fish farm: 16% change, $t = 0.51$, $df = 79$, $p > 0.05$; stormwater: -34% change, $t = -1.63$, $df = 79$, $p >0.05$; sewage: 8% change, $t = 0.36$, $df = 79$, $p >0.05$; Fig. 3b).

The recruitment of opportunistic species differed between the urban impacts. There were significantly fewer opportunistic species at marinas than controls (47% change, $t = -2.0$, $df = 80$, $p > 0.05$). In contrast, there were no detectable effects of fish farms (-11% change, $t = -0.77$, $df = 80$, $p > 0.05$), stormwater outfalls (-11% change, $t = -0.43$, $df = 80$, $p > 0.05$), or sewerage outfalls (-27% change, $t = -1.29$, $df = 80$, $p > 0.05$) on the number of opportunistic species relative to the controls (Fig. 4c). Nor were there detectable differences in the number of non-opportunistic species that recruited to pavers near fish farms (-33% change, $t = -0.77$, $df = 80$, $p > 0.05$), marinas (-58% change, $t = -1.79$, $df = 80$, $p > 0.05$), stormwater outfalls (14% change, $t = 0.36$, $df = 80$, $p > 0.05$) or sewerage outfalls (-20% change, $t = -0.54$, $df = 80$, $p > 0.05$) (Fig. 4d) relative to their control sites.

The total biotic cover was significantly higher on pavers near sewerage outfalls (72% increase, $t = 2.37$, $df = 79$, $p < 0.02$) relative to their paired control sites (Fig 4a). Fish farm pavers had slightly, but not significantly, higher biotic cover (46% increase, $t = 1.61$, $df = 79$, $p > 0.05$) than controls. There was significantly less abiotic cover on pavers at sewerage outfalls than at control sites (-79% change, $t = -3.47$, $df = 79$, $p < 0.001$), whilst those at marinas and stormwater drains tended to have slightly more, abiotic cover (21% increase, $t = 0.97$, $df = 79$ $p > 0.05$) and (62 % increase, $t = 1.75$, $df = 79$ $p > 0.05$), than the paired control sites (Fig. 4b).

Consistent with our expectations, there was 7.7-fold increase in the cover of non-indigenous species ($t = 2.04$, $df = 79$, $p > 0.001$), and 97% lower cover of natives ($t = -4.1$, $df = 79$, $p < 0.01$) at marinas than at the paired control sites, after accounting for random environmental variables (region, site and wave exposure), associated with the estuarine cline (Fig. 4c). Sewerage outfalls had a 12.8-fold higher cover of non-indigenous species ($t = 2.44$, $df = 79$, $p = 0.02$), but no significant difference in native cover ($t = 1.4$, $df = 79$, $p > 0.05$), compared to paired control sites.

In contrast, no detectable difference in cover of non-indigenous species (37% increase, $t = 0.9$, $df = 79$, $p > 0.05$) (Fig. 4c) or cover of native species (5% increase, $t = 0.24$, $df = 79$, $p > 0.05$) were found at stormwater drain sites relative to control sites. Fish farm pavers had significantly more (43%) native cover ($t = 1.97$, $df = 79$, $p = 0.05$) than at control locations but no detectable differences in the cover of non-indigenous species were found (66% decrease, $t = -0.71$, $df = 79$, $p > 0.05$). Overall, there were no detectable differences in the cover of non-opportunistic species between the fish farms (3% increase, $t = -3.0$, $df = 79$, $p > 0.05$), marinas (-100% change, $t < 0.01$, $df = 79$, $p > 0.05$), sewerage outfalls (46% increase, $t = 1.35$, $df = 79$, $p > 0.05$), and stormwater outfalls (-55% change, $t = -1.33$, $df = 79$, $p > 0.05$) (Fig.4f) relative to control sites.

Fish farms had a significant (85%, $t=2.38$ $df = 79$, $p < 0.05$) increase in cover of opportunistic species relative to their paired controls, as did sewerage outfalls (2.3-fold increase, $t = 2.04$, $df = 79$, $p = 0.05$) (Fig. 4e). The marinas (26% change, $t = 1.03$, $df = 79$, $p > 0.05$), and stormwater drains (34% increase, $t = 1.34$, $df = 79$, $p > 0.05$) (Fig.4e) had slight but non-significant positive impacts on opportunistic groups relative to their controls.

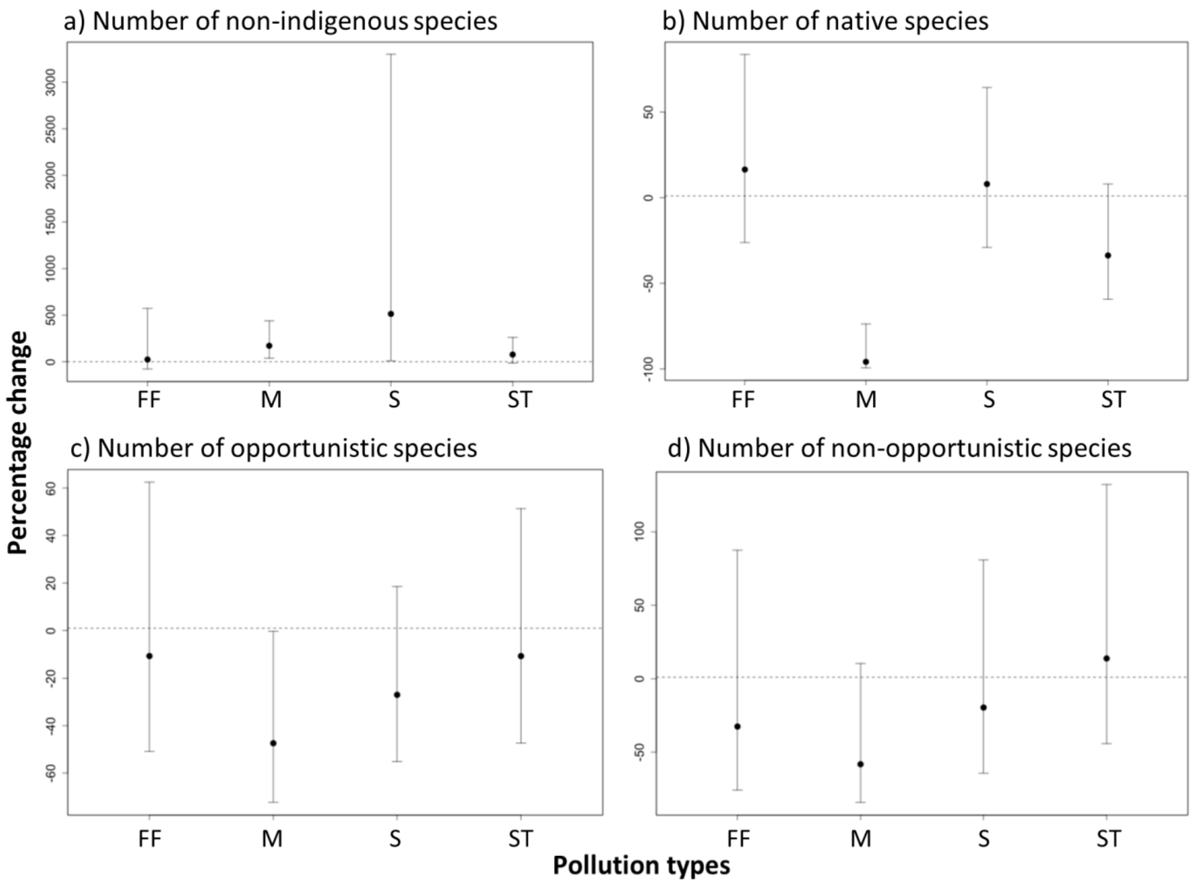

Figure 3: Effects of the urban pollution sources (fish farms (FF), marinas (M), sewerage outfalls (S), and stormwater drains (ST)) on the percentage change in the number of a) non-indigenous, b) native, c) opportunistic and d) non-opportunistic species recruiting to experimental pavers, relative to their associated control sites. Effects are significant if confidence intervals do not overlap zero. Percentage change was calculated from GLMM estimates based on a quasi-poisson, mixed effect model with a log link.

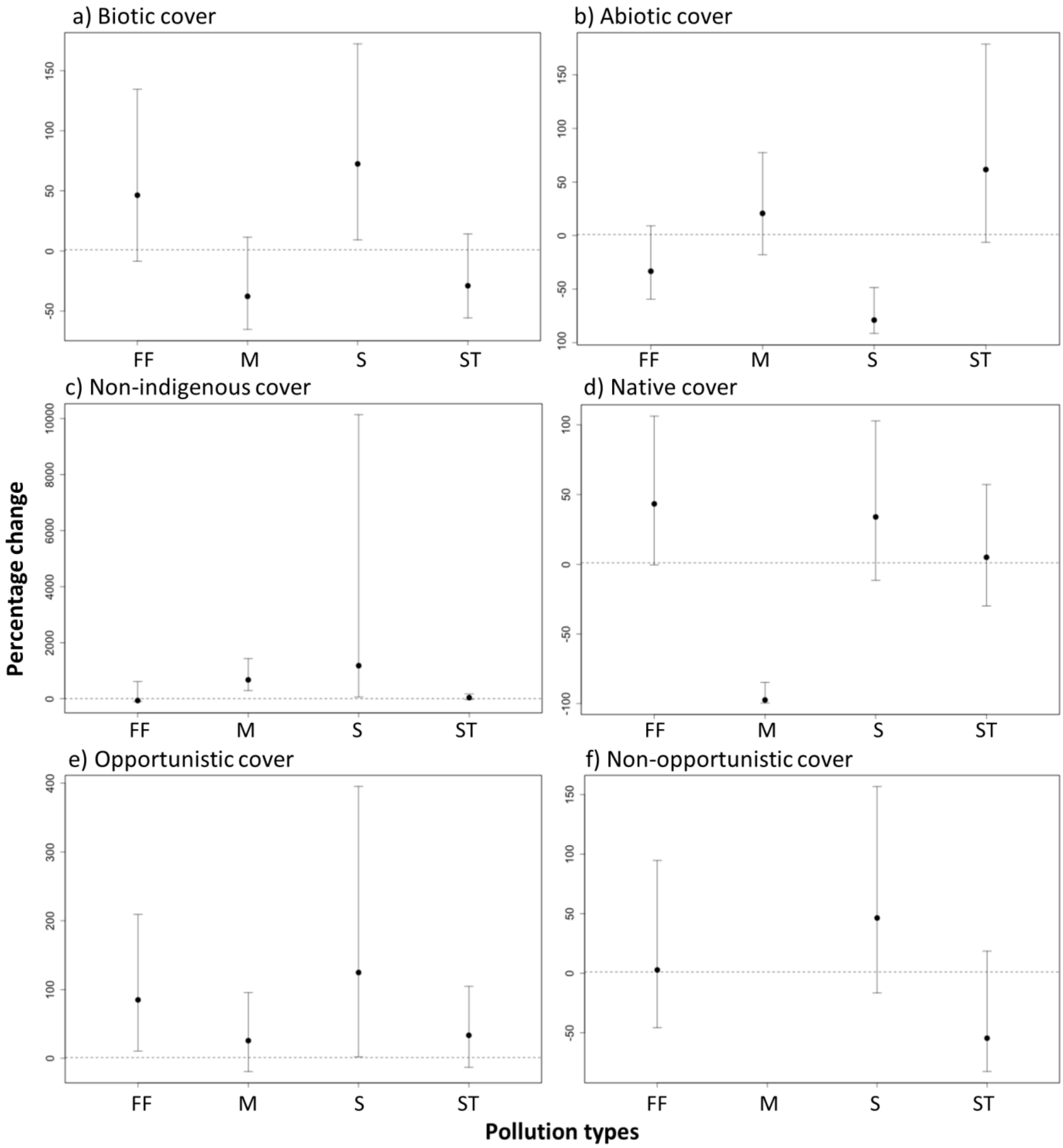

Figure 4: Effects of the urban pollution sources (fish farms (FF), marinas (M), sewerage outfalls (S), and stormwater drains (ST)) on the change in percentage cover of a) biotic species, b) abiotic (bare space and sediment), c) non-indigenous species d) native species, e) opportunistic species and e) non-opportunistic species recruiting to experimental pavers, relative to their associated control sites. Effects are significant if confidence intervals do not overlap zero. Percentage change was calculated from the GLMM estimates based on a quasi binomial, mixed effect model with a log link.

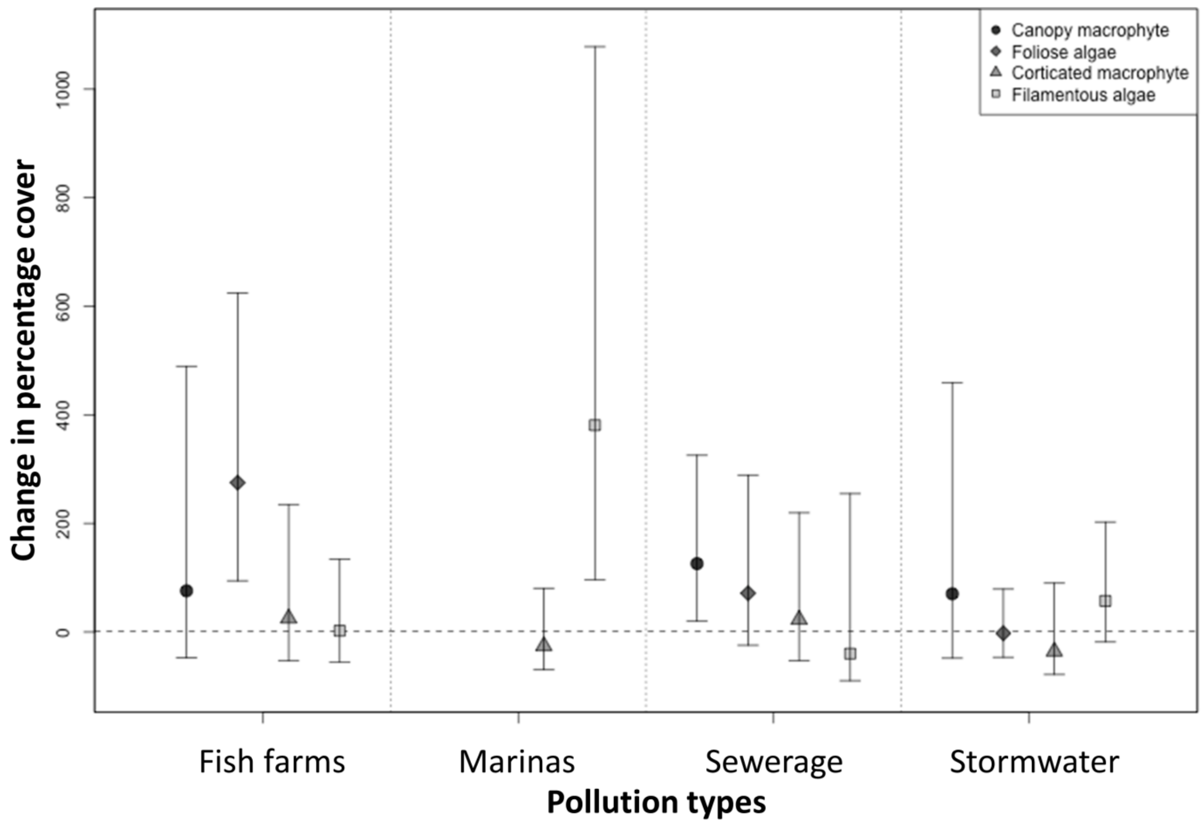

Figure 5: Variation in the cover of algal functional groups recruited to experimental pavers at pollution sources, relative to paired controls. Means and 95% confidence intervals are derived from linear mixed models of log response ratio against pollution types. Two marina plots are missing because algal groups were virtually absent from these areas.

Fish farms significantly enhanced growth of foliose algae (274% change, $t = 3.99$, $df = 79$, $P < 0.001$; Fig. 5), however no other algal groups were significantly different from controls. Very few algal species recruited to pavers placed near marinas, with filamentous algae the only functional group to recruit in greater density at marina sites relative to the control sites (381% increase, $t = 3.48$, $df = 79$, $P < 0.01$). Sewerage outfalls also had higher cover of canopy-algae recruits relative to the control sites (126% change, $t = 2.56$, $df = 79$, $P < 0.05$), but exerted no significant influence on other algal groups. No detectable effects of stormwater drains, region or wave exposure were found on the recruitment of algal groups.

Discussion

This is the first study to distinguish the effects of four different common urban impacts on the recruitment of algae and sessile invertebrates in a well-mixed estuarine ecosystem. Other studies have individually considered the impacts of marinas (Johnston et al., 2011) or artificial pilings (Hedge and Johnston, 2012; Johnston et al., 2011) on the nearby rocky reef at small scales; however, the design of this study allowed distinction of pollution impacts from estuary-wide natural variation. Our results support the growing body of literature that indicates that a range of urban impacts in estuaries (including marinas, sewerage outfalls, storm water drains and fish farms) can influence the establishment stages of sessile organisms on nearby rocky reefs (Airoldi and Beck, 2007; Gorman and Connell, 2009; Gorman et al., 2009; Mangialajo et al., 2008).

Marinas and sewerage outfalls consistently had greater recruitment of non-indigenous and cryptogenic species relative to their paired control sites. Anthropogenic structures such as marinas, wharves and jetties have been previously shown to harbour high densities of non-indigenous species (Carlton, 1987; Dafforn et al., 2009), including for mobile invertebrates in the Derwent Estuary (Ling et al., 2012). One hypothesis for this pattern has been that anthropogenic structures represent new substrate to colonise, without the resistance to invasion provided by established native communities (Mandryk et al., 2006).

The experimental pavers used in this study provided equivalent new substrate at locations across the whole estuary, yet those near the marinas (and sewerage outfalls) received clearly greater recruitment of non-indigenous species. This instead suggests that the increased settlement of non-indigenous species near marinas may be due to these structures providing source populations from which propagules of non-native species may spread to new areas (Williamson et al., 1986). An equivalent pattern is not suggested for mobile invasive species in this estuary, such as fishes and crustaceans, which instead appear to be capitalising on empty niches (Stuart-Smith et al 2015), but our results are supported by another study on sessile species that demonstrated high propagule pressure is a strong predictor of successful establishment of non-native species (Hedge and Johnston, 2012). This highlights potential differences in the mechanisms by which success of invasive species in disturbed habitats depends on the mobility of the species.

In our study area, the dominant non-indigenous species were *Codium fragile* subsp. *fragile* (Suringar) Hariot, 1889 [formerly *C. fragile* subsp. *tomentosoides* (van Goor) Silva, 1955] and *Polysiphonia* sp. *Codium fragile* subsp. *fragile* originated from Japan and Korea (Lyons and Scheibling, 2009), and has only recently been identified in South Australian and Tasmanian waters (McDonald et al., 2015). This species is found near marinas and sewerage outfalls because of its high tolerance of heavy metals and elevated nutrients, and ability to withstand sedimentation (Gorgula and Connell, 2004). It also regenerates and grows quicker than many native algal species (Bégin and Scheibling, 2003). The continuing spread and establishment of these algae in sites next to marinas and sewerage outfalls is especially concerning given the stress already placed on the native biodiversity by these sources of contamination (Vye et al., 2015).

Polysiphonia species were also found next to marinas and sewerage outfalls. The introduced red filamentous alga *Polysiphonia senticulosa* occurs in moderate water movement, an important trait for establishing permanent populations in newly invaded habitats (Thomsen et al., 2007). *Polysiphonia* species have primarily been recorded on artificial structures in marinas and ports (Womersley, 2003), suggesting it may be quick to establish on newly available substrates, but is perhaps less able to compete with native communities (even if moderately impacted by stressors), when present. It is likely also tolerant of contamination by heavy metals and other pollutants. In this study it was able to colonize free space more efficiently than any native species.

Similar to other studies, a greater cover of opportunistic and foliose species such as *Ulva rigida* was present near sewerage outfalls and fish farms relative to control sites (Lavery and McComb, 1991; Munda and Veber, 2004; Oh et al., 2015). These algae are ephemeral, with the ability to assimilate high levels of nutrients, reproduce all year round, and tolerate excess sedimentation (Correa et al., 1999; Littler and Murray, 1975). Contrary to our expectations, recruitment of native algae (*Carpoglossum confluens*, *Ulva rigida*, *Acrocarpia paniculata*) was high at sites near sewerage outfalls relative to control sites. Elevated nutrient inputs may allow native taxa to also colonise larger areas of nearshore reefs (Campbell, 2001), where other pollutants do not prohibit colonisation or survival.

Heavy metal pollution and biocides associated with marinas are known to dramatically change community structure on marine hard substrata (Dafforn et al., 2009; Johnston et al., 2011). We found that sites near marinas had half the number and cover of native species relative to their controls. This may not only be a direct result of decreased post settlement performance due to these contaminants, but also possibly through interactions between contaminants, elevated turbidity, and sediment loads. The lack of native recruits to these areas may translate to major changes to the ecosystem, resulting in facilitation of the dominance of more tolerant exotics (Stachowicz et al., 2002). In addition, copper in antifoulant paints from boats may reduce native species recruitment success, while high vessel densities may simultaneously result in increased transport of non-indigenous species into these disturbed estuarine areas (Dafforn et al., 2008; Piola et al., 2009; Piola and Johnston, 2008a; Piola and Johnston, 2008b).

Assessing the impacts of urbanisation of benthic communities in estuaries is complicated by seasonal changes, small-scale spatial variability (Chapman, 1995), and the interactions of several chronic stressors (Chester et al., 1983a; Smale, 2012; Underwood and Kennelly, 1990). Estuaries are naturally stressed due to variations in salinity, organic loading, sediment stability and oxygen concentrations (Tweedley et al., 2015). Consequently, detection of impacts requires a signal above and beyond noise from the background of natural variability (Veríssimo et al., 2013). By separately partitioning the effects of estuary region and wave exposure our statistical models, we detected a clear signature of pollution over natural causes of variability.

Ubiquitously poor water quality and elevated nutrient concentrations across human-dominated coasts may also make impacts more difficult to detect than in pristine areas (Russell et al., 2005). As in chapters 2 and 3, it was difficult to establish adequate controls in the upper estuary, or to unambiguously isolate the particular impacts of different pollutants from general urban impacts. Through use of models where natural factors associated with the estuarine gradient are included, the urban impacts that we were able to detect in the Derwent estuary are conservative estimates of true effects.

Sources of stress in estuaries, such as an increase in the number, frequency, and residency times of visiting vessels (Dafforn et al., 2009; Schiff et al., 2004), and further nutrient enrichment from sewage and agricultural sources, will likely result in expansion of pollution-tolerant opportunistic species, in turn resulting in major habitat change (Simberloff and Von Holle, 1999). In order to manage current urban impacts and prevent detrimental changes to native community structure and dynamics, current pollutant loads need to be reduced, and pollution impacts prevented where not yet present. Improved water quality may also be achieved through the adoption of nontoxic antifoulants, diversion of stormwater drains through wetlands for filtration, and a reduction of sewage waste released into estuaries. Some finfish farming companies have begun phasing out copper-based antifoulants, but toxic antifoulants are still being used, and the magnitude of impacts observed in marinas suggests that regulations relating to antifoulants used by recreational vessels may require further investigation. Novel engineering techniques could be used to increase habitat for native marine species (Firth et al., 2013). Remediation of contaminated sediments is necessary to avoid the risk of toxicant release during resuspension events (Beck, 1996). On the other hand, the intrinsic characteristics of locations also determine if an area is more or less prone to invasion. High flow and high wave energy environments allow more efficient dispersal and removal of pollutants than sheltered environments, and thus are likely to require less management intervention in the long-term.

Conclusions

Our study indicates that recruitment of sessile organisms is affected by pollution in different ways. Pollution from marinas and sewerage outfalls resulted in an increase in the proportion of non-indigenous taxa amongst recruits. Marinas caused a significant reduction in both number and cover of native species, whereas non-indigenous species and filamentous species increased. Sewerage outfalls promoted the recruitment of both non-indigenous and canopy species, an unusual mix. Fish farms and sewerage were associated with an increase in opportunistic algae, driven by foliose macro-algae. Pollution clearly increases the potential for negative shifts in rocky coastal habitat from native to more opportunistic/non-indigenous species, in part by disproportionately reducing the recruitment of native species, potentially allowing more invasive species to recruit in empty niches in locations where propagule pressure is greatest.

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Chapter 5. Interactive responses of primary producers and grazers to urban pollution on estuarine rocky reefs

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Abstract

Primary producers in estuaries are controlled by interactions involving anthropogenic stressors, environmental conditions, and ecological processes such as grazing. Using a paired impact-control experimental design, we tested the strength of interactions between common pollution sources (fish farms, marinas, sewerage and stormwater), algae and grazers (macro- and meso-fauna) on rocky reefs in a highly urbanised estuary in Tasmania, Australia. Algal assemblages were directly affected by pollution impacts, with grazers having little influence on transplanted algae. Leathery algae enhanced mesograzer abundance. Mesograzers were negatively influenced by marinas but positively associated with fish farms. Some mild interactions were inferred in which grazers had negative effects on filamentous but positive effects on foliose algal recruits. Overall, direct algal responses to pollution (bottom-up processes) appear to predominantly drive trophic and community structure in this urban estuary.

Keywords: benthic, temperate, epifauna, macrograzers, mesograzers, urbanised estuary, herbivory, pollution, macro algae

Introduction

Urban pollution profoundly influences community structure in coastal areas (Airoldi and Beck, 2007), through both direct and indirect effects on assemblages (Underwood and Fairweather, 1989). This is particularly the case in human-dominated sheltered embayments and estuaries, which are stressed by a variety of urban as well as natural environmental pressures (Edgar et al. 2000). Common sources of urban pollution (such as marinas, stormwater drains, sewerage outlets and fin-fish aquaculture) release heavy metals, toxicants, nutrients and sediment into the water column that create impacts at multiple trophic levels (Johnston et al., 2011; Pratt et al., 1981; Russell et al., 2005; Soto and Norambuena, 2004). These pollutants can influence reef assemblages and alter food webs differently, potentially altering the importance of bottom–up versus top–down forcing, especially in naturally complex ecosystems (Duffy et al., 2015). Assessing the effects of common pollutants at multiple trophic levels is important for separating the effects of urban impacts and environmental and ecological drivers of estuarine biodiversity patterns.

While the preceding chapters indicate that algae respond selectively to different pollution sources, they do not elucidate whether algae are impacted directly or effects are mediated indirectly through ecological interactions, such as herbivory. Herbivory can be an influential driver of algal community structure in marine systems (Franco et al., 2015; Poore et al., 2009), and has been termed a 'top-down" control. Grazers eat and inhabit thalli of macroalgae, and have the capacity to suppress growth and modify ecosystem processes over large areas (Vanderklift et al., 2009). Consumption of algae by grazers varies with algal traits and type of herbivore (Poore et al., 2012), and may be enhanced by pollution, particularly nutrient loadings in urban areas (Cloern, 2001). In the absence of herbivores, there is a greater productivity response to nutrient enrichment (Burkepile and Hay, 2006). Pollution might also influence grazers and thus grazing-algae interactions.

Macrobenthic invertebrates are considered to be powerful indicators in the assessment of ecological conditions (Lavesque et al., 2009) and can have a strong influence on macroalgae. In temperate areas, rocky shore mobile macroinvertebrates (echinoderms, large gastropods and large crustaceans >2.5 cm length) and cryptic fishes are abundant and diverse on algal dominated rocky reefs (Edgar, 2008). The larger grazers can alter physical structure and productivity of macroalgae (McCormick and Stevenson, 1991), in some situations eroding the resilience of kelp beds via direct physical effects (Wernberg et al., 2011). Occasionally "good grazers go bad" (Schiel and Foster, 2015), such as when densities of native species such as gastropods or urchins are excessively high relative to food sources. Urbanised reefs often have high abundances of herbivorous sea urchins (e.g. *Heliocidaris erythrogramma* near the major urban centres in Port Phillip Bay; Stuart-Smith et al., 2015), yet whether pollution facilitates increased densities and overgrazing by sea urchins and invertebrates remains unknown.

Mesograzers (isopods, amphipods and small gastropods) are highly abundant in macroalgal beds (Poore et al., 2009). They comprise important prey for fishes (Edgar and Shaw, 1995), contribute to trophic cascades (Duffy et al., 2005), and respond to, and can also affect, algal structure and biomass (Duffy and Hay, 2000). For example, amphipod grazing has been associated with declines in biomass of kelp (Graham, 2002) and seagrass beds (Whalen et al., 2013). Mesograzers can also facilitate the dominance of habitat-forming macrophytes by grazing competitively-superior epiphytic algae that reduce light availability (Whalen et al., 2013), and through this process may increase resilience in reef ecosystems (Scheffer et al., 2001). In some situations, they can also counter the effects of resource enrichment, compensating for minor to moderate nutrient loadings by consuming the additional productivity of opportunistic species (McSkimming et al., 2015). In response to herbivory, macroalgae can use anti-herbivore defences to deter consumers (Hay 1996, Cronin, 2001).

Different pollution types may influence the intensity of grazing through a number of mechanisms, including changed behaviour of grazers (changing the dominance of feeding groups), mortality of grazers, alteration to settlement and recruitment of both grazers and algae, or by influencing the type and availability of food sources (Dayton et al., 1998; Pearson and Rosenberg, 1978). A change in the intensity of grazer pressure can lead to substantial changes in the reef community structure (Ling et al., 2008; Ling et al., 2015), potentially contributing to habitat loss in urbanised areas.

Pollution can also influence palatability of the algal community by altering the composition of species, but questions remain as to whether algal form or chemical strength is a better defence against consumption (Koricheva, 2002). Prior studies have used traits associated with thallus morphology and toughness to predict responses to herbivory. Large tough algae and crustose forms are widely considered to be most resistant to grazers (Steneck and Watling, 1982), whereas a recent meta-analysis suggests that Fucales, Ulvales and Laminariales suffer most from herbivory, and that algal traits, including taxonomic composition and morphology, are largely responsible for highly variable grazing impacts on algal assemblages (Poore et al., 2012). Traditionally, opportunistic species were thought to be more vulnerable to herbivory than tough or chemically-defended algae, due to limited investments in chemical or structural defences (Littler and Littler, 1980). Palatability within the algal community certainly plays an important role in regulating patchiness of different forms, and the feeding behaviour of grazers, and thus may shape urban seascapes.

Clearly, an improved understanding of grazer-algal interactions is needed because grazers can have a profound influence on community structure and, therefore, ecosystem processes. While some studies have demonstrated important roles of marine grazers, relatively few have contrasted different groups of grazers (e.g. Carpenter and Lodge, 1986) or incorporated natural variation in environmental factors (Duffy et al., 2015). A large body of literature has focused on macrograzer effects, yet the strength of mesograzer effects on algal hosts and vice versa, are rarely quantified (Poore et al., 2009). In particular, little is known about interactions of common pollution types and grazing on macroalgal communities.

The present study builds on an investigation of the persistence and recruitment of algal communities when transplanted algae are placed in close proximity to pollution sources (Chapters 3 and 4). Healthy transplanted algal communities exhibited a rapid negative response to pollution impacts. Fish farms, marinas and stormwater had a strong positive effect on cover of filamentous algae, whereas marinas significantly depressed canopy algae and foliose algae, and sewerage outfalls had little apparent effect. Sessile biota recruiting to clear space (bare pavers) near these same pollution sources responded slightly differently than transplanted algae. Fish farms significantly enhanced the recruitment of foliose but not filamentous algae (chapter 4). Recruitment of canopy algae increased near sewage outfalls, whilst filamentous and non-indigenous algae increased near marinas, but virtually no recruitment of native macroalgae occurred. These studies of persistence and recruitment indicated that pollution enhances the abundance of non-desirable species, and that marinas had the most pronounced negative effects on native biotas.

We hypothesised that different algal functional groups, with distinct physiologies, would show contrasting responses to grazing and pollution. We considered three specific questions:

1. How do common pollution sources influence grazers and algal groups?
2. Are meso- and macro-grazers differently influenced by pollution?
3. Is pollution changing the palatability of urban habitats?
4. **Are** macroalgae indirectly affected by pollution types through the interactive influences of grazers?

Methods

Study region

The study was undertaken in the Derwent Estuary and the D'Entrecasteaux Channel (Fig. 1), a conjoined estuarine system exposed to moderated oceanic swell with wide entrances that promote efficient marine flushing (Whitehead et al., 2010). The Derwent Estuary is a drowned river valley that extends inland for 52 km with a total catchment area of 198 km^2 (Coughanowr, 1995). It is a salt-wedge estuary that is well mixed in the middle and lower regions. Freshwater predominantly flows downstream along the eastern shore as a result of prevailing westerly winds (Butler, 2006). Approximately 202,000 people (around 40% of Tasmania's population) live around its shores. The land adjacent to the middle estuary supports large industries, including paper production, zinc smelting, fertilizer production and boat building (Coughanowr and Whitehead, 2013a). The adjacent D'Entrecasteaux Channel is located between the mainland of Tasmania and Bruny Island. It is a 40-km long semi-enclosed water body, with a net flow of seawater from the D'Entrecasteaux Channel into the mouth of the Derwent (Whitehead et al., 2010). It has a high concentration of fish farms (Oh et al., 2015). Both estuaries are fringed by extensive subtidal rocky reefs.

Study sites

We conducted a manipulative experiment on subtidal estuarine reefs, with impact sites located in close proximity to four types of pollution sources. The impact sites were compared to control sites that were located on rocky reefs at a distance of 1-3 km. Marina sites were located <50 m from boat moorings inside marinas harbouring 200-400 recreational and commercial vessels. Fish farm sites were located <100 m from farm lease boundaries. Leases typically had 4-8 cages (20-30 m in diameter), which were periodically moved within the lease. Stormwater sites were located <20 m from the outlets of large concrete pipes (30-50 cm in diameter). Sewage sites were within 20 m of sewerage pipe outfalls. Experiments commenced in March 2010 and ended in March 2011.

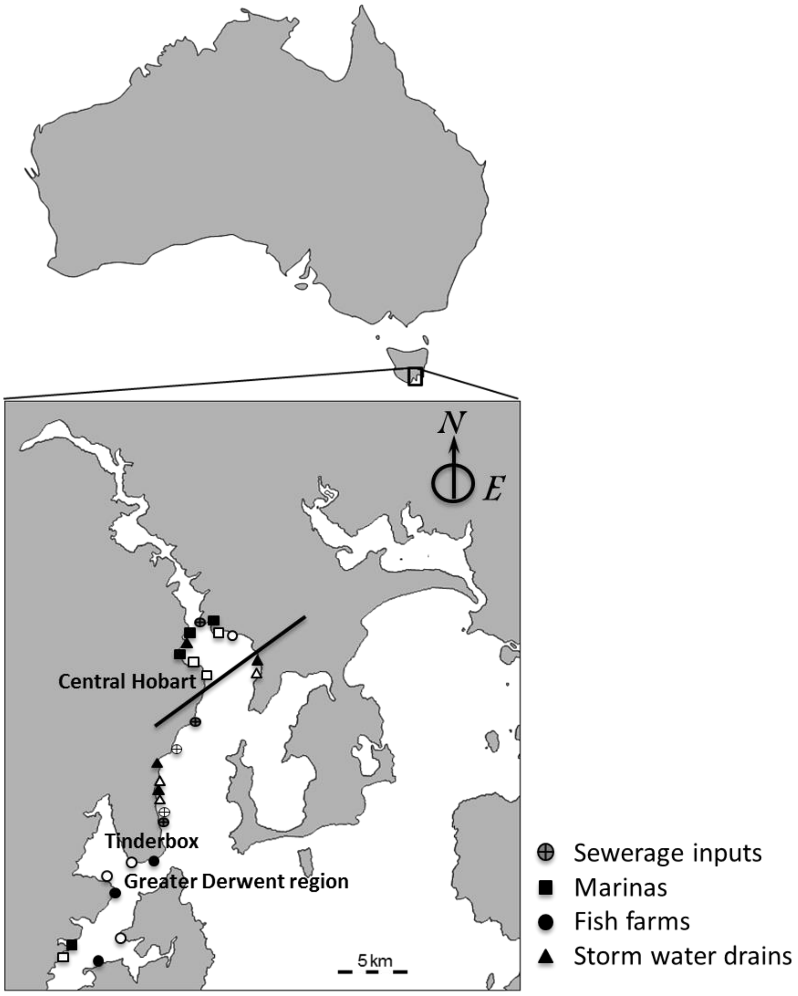

Figure 1. Location of the 27 sites used to test the effects of different urban impacts on the cover of the macro-algal and sessile invertebrate recruits in the Derwent and D'Entrecasteaux Channel, Tasmania Lat: -43.03 Long: 147.34. Solid line indicates sites within 5 km from the city. Solid symbols are the impact sites, open symbols are control sites.

Algal communities

Cement pavers were used to standardise the macroalgal habitat and to test for effects of different pollution types. Initially one hundred and thirty eight large garden pavers (300 x 300 x 50 mm), were deployed at Tinderbox (-43.03°, 147.34°), a central and comparatively well-flushed and less impacted location near the mouths of the estuary systems (Fig 1.). Half of each paver was covered with plastic to prevent recruitment. Pavers remained submerged at 3-4 m depth for seven months, during which time a diverse community developed on the uncovered part of the paver, including perennial k-reproductive strategy species (Chapter 3).

Four replicate pavers and their associated macroalgal assemblages were transplanted to areas subject to each of four types of pollution (marinas, stormwater discharge, sewage discharge and fish farms) and another four pavers transplanted to each of the paired control locations. Thus, pavers were set out at 32 sites originally, however due to overturning and movement by storms, only 27 sites had usable pavers at the conclusion of the experiment. The position of the pavers was chosen randomly within 2-4 m depth. Control pavers were placed on a reef 1 km from pollution sources. The plastic paver wrapping was removed to expose a clean surface across half the paver. Pavers were horizontally oriented and positioned ~ 1 m apart, at least 3 m from naturally-occurring reef edge and sand scour.

The composition of transplant and recruit algal communities were assessed after one year to determine the presence and relative abundance of macroalgal species. One 300 x 300 mm quadrat was placed on each paver (recruit and established algae) to delimit the sampling surface. String divided the quadrat into a grid. On each side of the paver, uncovered and previously covered, under 18 intersection points the algal taxon was identified and the total cover was estimated separately for different structural layers in order: epiphytic algae, canopy algae, foliose algae, turf algae and encrusting organisms. Point counts of sessile organisms (algae and sessile invertebrate) were recorded on an underwater slate. The majority of species were recorded to species level. Other taxa were recorded at the highest taxonomic resolution possible. Abiotic variables (sediment and bare rock) were excluded from the community analysis, and biotic factors were recalculated to 100% percentage cover. As a quality-control measure, a photograph was taken 0.5 m above each block. These photos and collection of specimens near pavers allowed later identification of species that could not be identified in the field.

Mesograzer community

Amphipods and other small invertebrate grazers were investigated using standardised rope fibre habitats, which were placed in the field 12 weeks prior to collection of pavers. Each habitat was made from 50 g of 100-mm long tanikalon rope fibre tied to bricks to mimic filamentous algae, a technique that has been used previously (Edgar, 1991; Edgar and Klumpp, 2003). Three replicate rope fibre habitats were placed on rocky reefs within 2 m of pavers at each of the four types of impact and paired control locations.

Invertebrates associated with rope fibre habitats (hereafter mesograzer) were sampled by detaching habitats from bricks, and enclosing within plastic bags. Contents of bags were preserved using formalin and returned to the laboratory, where invertebrates >0.5 mm sieve size were extracted by washing samples over a stacked series of sieves (Edgar, 1990). Material retained on each sieve was sorted to species wherever possible and counted under a dissecting microscope.

Macrofaunal community

Cryptic mobile macro-invertebrates and cryptic fishes were quantified under each paver after one year. After algal taxa had been recorded, each paver was turned to assess macro-fauna on the underside of the paver and in the paver footprint, the area directly beneath the paver. This technique primarily revealed echinoderms, decapods, and large-footed molluscs, such as abalone and chitons (Alexander, 2013). Species were identified and counted *in situ*. Where the identity of a species was uncertain, photographs were taken for later verification. When present in high numbers, crustaceans (particularly the abundant New Zealand half-crab *Petrolisthes elongatus*) were estimated rather than counted.

Functional groups and palatability assessment

Algal groups were measured by percentage cover. Algae were categorised using functional groups defined by Steneck & Dethier (1994) and details therein, in order to assess their response to different types of pollution (Burkepile and Hay, 2006) and associated morphological properties, which make them more or less susceptible to grazing. We also wanted to compare functional algal groups with the recent findings of Poore et al. (2012) who found that grazer impacts can be predicted by producer traits. We created a palatability index based on this paper (Fig. 4b in Poore et al., 2012). The palatability of each assemblage under urban impacts and paired controls was calculated by multiplying the mean palatability of each algal order (Fig. 4b in Poore et al., 2012) on the paver by the cover of that algal order. These values were summed then divided by total cover to represent the mean palatability of taxa on the paver.

Different macrofaunal feeding strategies can contribute to different ecological outcomes via morphological and behavioural properties (Peng et al., 2013; Cummins et al., 2005). In this paper we considered total macrograzers to include species that could feed on living algae, and macrograzers were only herbivores. We consider mesograzers to include species that directly feed on living attached algae, detritus and microphytobenthos (Graham 2008).

Statistical analysis

Univariate analysis

To quantify the effect of pollution on algal and grazer groups, we applied mixed effect generalised linear models using R (R Core Team, 2014) and the statistical package piecewiseSEM (Venables and Ripley, 2002). A quasi-poisson distribution was used for the abundance data (macro/mesograzers and macrofaunal feeding strategy groups) and a quasi-binomial distribution for algal cover data. A log link function was employed for both data types, so that the estimates can be presented as percentage change relative to the controls. For the palatability index data, linear regression was used. The basic unit was at the replicate paver level.

The fixed effects in the model were impacts (five levels: fish farms, marinas, sewerage outfalls, storm water drains and controls), estuarine area and wave exposure. Along with estuarine area and wave exposure, a random effect of site was included in the model to adjust for the natural variability between sites. As each impact site and its paired control formed one site, the analysis can be regarded as a block design. Estuarine area categorically distinguished sites within 5 km of the capital city, Hobart, from the downstream Derwent region (> 5 km, Fig. 1). Sites near the capital city were assumed to be more heavily impacted due to cumulative pressures (Chapter 3). Wave exposure was quantified using a fetch model, which uses distance to coast in multiple directions as a proxy for potential wind-generated waves (Hill et al., 2010).

The 95% confidence intervals (CIs) and p-values were obtained for the model estimates (or log response ratios), assuming a t-distribution with the appropriate degrees of freedom. For visual display, log response ratios of abundance/cover for the urban impacts (and their CIs) were converted to percentage change relative to controls, as 100*(exp(estimate)-1). CIs and p-values were used to assess whether there was a significant of impact of each pollution type relative to controls, on the abundance or cover of the different functional groups.

Structural Equation Modelling

Structural equation modelling (SEM) was used to disentangle complex interactions among a hypothesized series of cause-effect relationships between pollution types, environmental variables, mesograzer and macrograzer, and algal functional groups (Fig. 2). A piecewise SEM was employed to map the interconnected paths of the ecosystem using a series of multiple regressions. The model network is used to evaluate the strength of effects, allowing for identification of important indirect pathways (Lefcheck, 2016).

The grazer/pollution model was constructed based on the conceptual model shown in Fig. 2, with pollution type, environmental variables, macrograzers and mesograzers influencing the cover of the algal group, and algal group also influencing mesograzers. Pollution variables were expected to affect the algal functional groups directly and also indirectly through variations in mesograzer and macrograzer abundances.

In our model we considered pollution impacts to have bottom up effects on algal groups, macrograzers to have top down effects on algal groups, and algal groups to influence mesograzer abundance. With this generalised conceptual model (Fig. 2) and variation therein, we tested each algal functional group separately.

Some pollution impacts were hypothesised to make algae more palatable (Duffy 2002), which in turn may affect the reef habitat. Transplant and recruit algal communities were considered separately as persistence and recruitment responses to grazing may differ. Algal recruits are widely considered to be more susceptible to grazing than established macroalgae (Lotze 2000). SEMs were run separately on four different algal groups (filamentous, foliose, leathery and total algae) for both transplant and recruit communities.

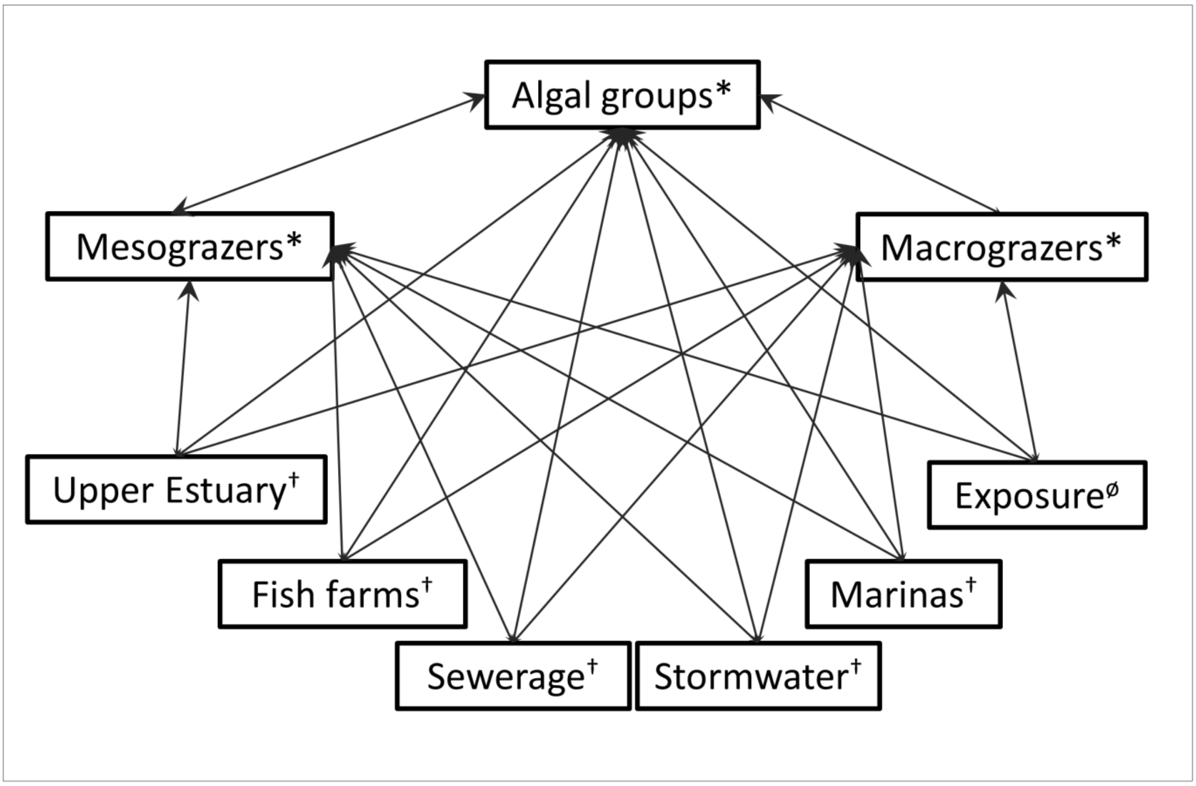

Figure 2: Grazer/pollution model: Arrow direction shows the causal effect hypotheses of biotic and abiotic variables in relation to algal group. Each algal group was analysed separately. *, † and ø indicates biological, anthropogenic and environmental variables respectively.

Each algal group SEM consists of three regression models, where the outcome variables for each model are abundance of mesograzers, macrograzers and cover of that particular algal group. Individual model fits for each of the linear regressions within the SEMs were assessed by calculating pseudo R^2. We transformed the abundance data ($\log_{10}$) and the cover data (arcsine) to meet the assumptions of homoscedasticity and normality for the linear regression models. Estuarine area and exposure were treated as fixed effects and site was treated as a random effect in the models. Analyses were run in R version 3.1.0 (R Core Team, 2014) using the statistical package piecewiseSEM (https://github.com/jslefche/piecewiseSEM) (Lefcheck, 2016).

Although the directions of the SEM pathways are fixed for each model fitting, the direction of arrows can be reversed in model comparisons to assess the direction of causality. Given uncertainty about whether mesograzers would primarily respond to, or influence, algal cover, initial model comparisons tested the direction of arrows between these two variables through identification of the model with lowest Akaike Information Criterion (AIC). A better fit was found for the models where the arrows were directed from algal cover to mesograzer, consequently these models are presented in the results.

Results

Overarching biotic response

Total macrograzers and mesograzers

Macrograzers exhibited no significant response to fish farms ($t = 1.35$, $df = 79$, $P > 0.05$), marinas ($t = 1.07$, $df = 79$, $P > 0.05$), sewerage inputs ($t = 0.09$, $df = 79$, $P > 0.05$) or stormwater drains (Fig. 3, graph (a), $t = 1.92$, $df = 79$, $P > 0.05$). Mesograzer abundance was significantly higher at stormwater drains (Fig. 3, graph (a), 93% increase, $t = 2.23$, $df = 54$, $P < 0.05$) than control sites. Of all the pollution types, the strongest effect was found at marinas, where mesograzers were much less abundant than at controls (-74% change, $t = -3.82$, $df = 54$, $P < 0.001$). Fish farms and sewage outlets had no significant effect on mesograzers. Both estuarine area (109% change, $t = 2.03$, $df = 12$, $p < 0.05$) and exposure ($t = 2.09$, $df = 54$, $P < 0.05$), significantly affected mesograzer abundance, but macrograzers were unaffected ($t = 1.09$, $df = 54$, $P > 0.05$).

Total transplanted and recruit algal cover

Total cover of transplanted algae showed no significant response to sewerage and fish farms (Fig.3, graph (b), 15% change, $t = 1.32$, $df = 79$, $P > 0.05$ and -3% change, $t = -0.26$, $df = 79$, $P > 0.05$), however the total cover of recruited algae was significantly higher for both fish farms (60% change, $t = 2.03$, $df = 79$, $P < 0.05$) and sewerage outfalls (77% change, $t = 2.46$, $df = 79$, $P = 0.02$). At marinas, total cover of transplanted algae showed a 48% decrease in cover compared to control locations ($t = -4.75$, $df = 79$, $P < 0.001$), while recruited algae did not change relative to the controls. Stormwater caused no significant change in cover for either transplants or recruits relative to controls. The environmental variables, estuarine area and wave exposure did not significantly affect total cover of transplants or recruits.

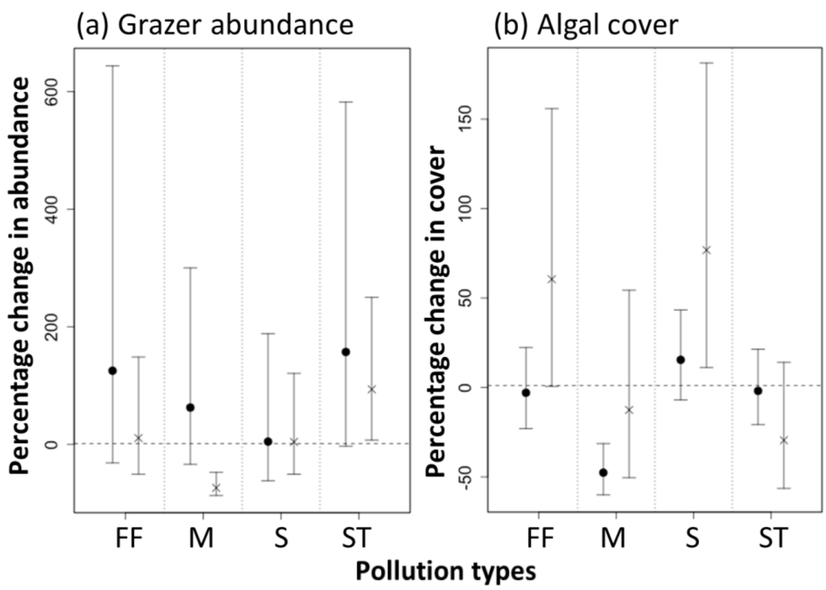

Figure 3. Percentage change in (a) grazing invertebrate abundance (macrograzers - black circles; mesograzers – crosses) (b) total algal cover at fish farms (FF), marinas (M), sewerage outfalls (S) and stormwater outlets (ST), relative to associated controls sites. Total cover of transplanted algae is shown using black circles and recruited algae using crosses. %change in abundance/cover and 95% confidence intervals are derived from linear mixed models of log response ratio against pollution types. The dotted line at zero represents no effect. Note the differences in the y-axis scales.

Palatability

The palatability of transplanted algal communities near urban pollution sources did not significantly differ from values at control sites for any impact type (Fig. 4a, Table 3). Areas near central Hobart and with lower wave exposure had less palatable algae ($t = -5.44$, $df = 12$, $P < 0.001$; and $t = -3.84$, $df = 75$, $P < 0.001$, respectively). The palatability of recruit communities was greater at fish farms than their controls ($t = 2.05$, $df = 60$, $P < 0.05$), (Fig 4b), but there were no effects for the other impact sources (Fig. 4). Algal recruit palatability was not influenced by estuarine area and wave exposure (Table 3).

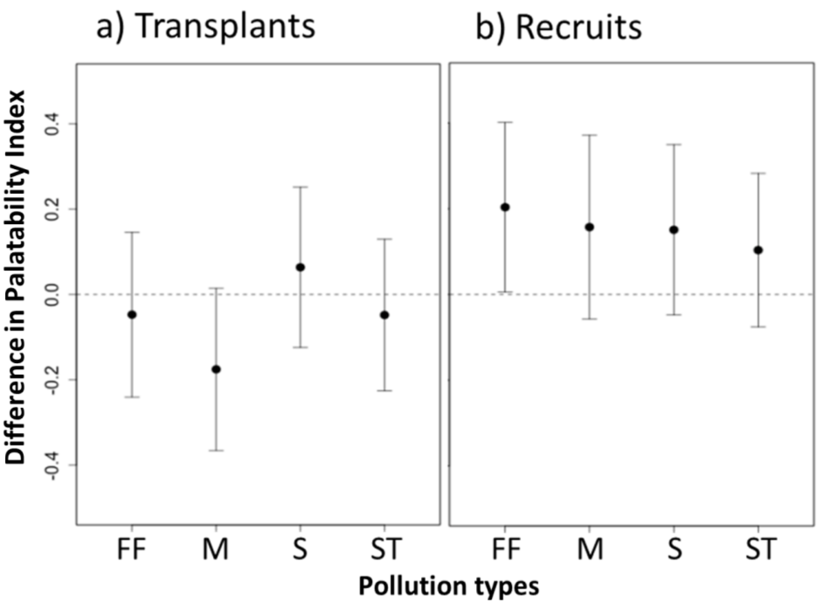

Figure 4. Effects of fish farm, marina, sewerage and stormwater impacts on the palatability of a) transplant algae and b) recruited algae, relative to their controls. The mean difference in palatability and 95% confidence intervals are derived from linear mixed models of log response ratio against pollution types. The dotted line at zero represents no effect. Fish farms = FF, marinas = M, sewerage = S, storm water drains = S

Table 3. Effects of pollution types and environmental variables on algae transplant and recruit palatability index (**0.01< P < 0.001). Significant values are in bold. A Kruskal-Wallis Test was applied.

Response	**Fish farm**	**Marina**	**Sewerage**	**Storm water**	**Region**	**Exposure**
Transplant	-0.49	-1.84	0.67	-0.54	**-5.44****	**-3.83****
Recruits	2.05	1.46	1.51	1.15	-1.25	0.83

Transplant community structural equation models

All of the biological variables were influenced by environmental and pollution variables in slightly different ways (Fig. 5). Filamentous algae increased with exposure, proximity to the central city, stormwater drains, fish farms and marinas, shown as solid black lines on Figure 5a (supplementary material; Table 1). Marinas had a positive effect on the abundance of total macrograzers. Foliose algae had higher abundances in the upper estuary area of the Derwent, however marinas had negative effects on foliose algal cover (Figure 5b) and also on abundances of mesograzers. In contrast, fish farms had a positive effect on abundances of mesograzers (supplementary material; Table 2).

Leathery algae were less abundant in areas of exposure and the urban region (Figure 6c, Supplementary material; Table 3). Fish farms had a positive effect on mesograzer abundance. Mesograzers were strongly positively influenced by leathery algae (Figure c, supplementary material; Table 3). Total algal cover was strongly negatively affected by marinas (Figure 6d, Supplementary material; Table 4).

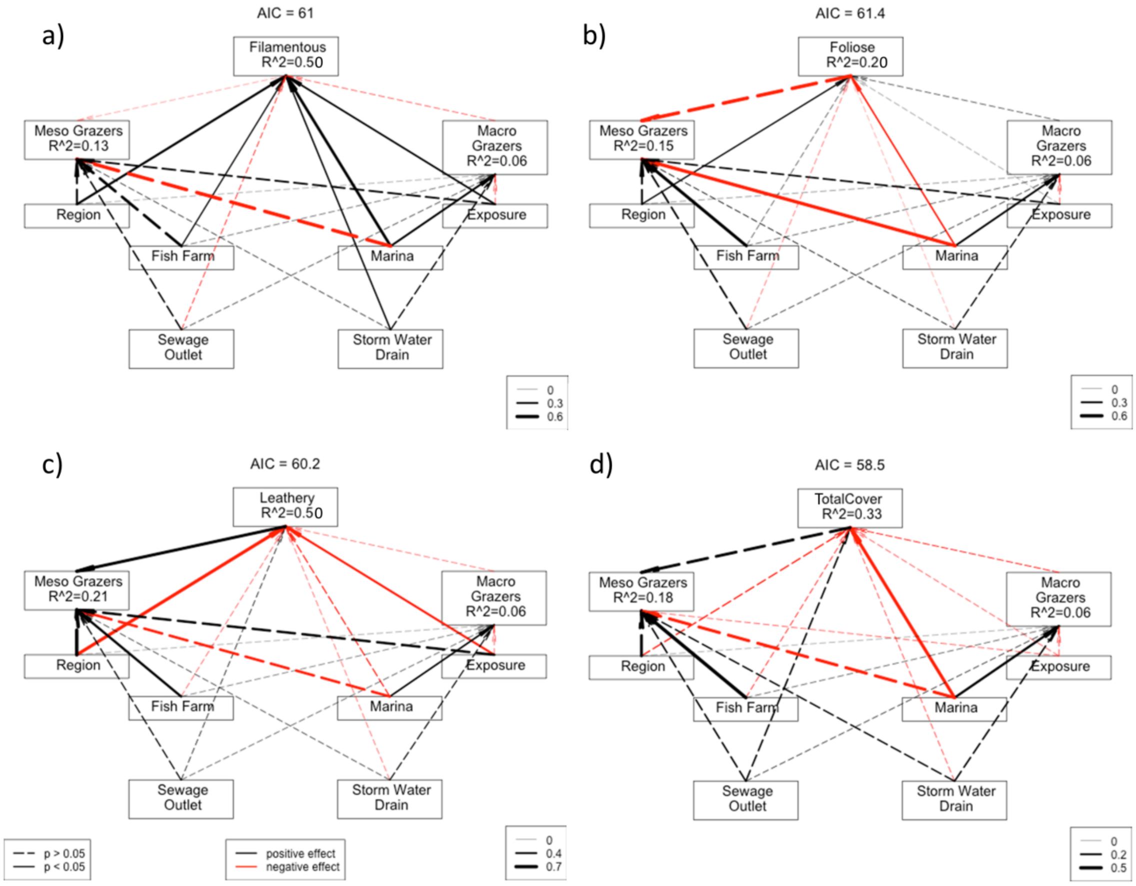

Figure 5. Structural equation model outcomes relating environmental and biological variables to cover on transplanted pavers for a) filamentous algae; b) foliose algae; c) canopy algae; d) total algal cover. Solid black lines represent significant positive pathways, while solid red lines represent significant negative pathways. Line thickness is scaled to match the standardised regression coefficients, with greater thickness indicating a stronger relationship. Dashed lines indicate non-significant (but tested) pathways.

Recruit community structural equation models

Overall recruitment data were more patchy and variable than transplant data, consequently algal responses are less well explained (i.e. R^2 values lower), however similar patterns were evident. Marinas had a positive effect on filamentous algal establishment and macrograzers, but a very strong negative effect on mesograzers as shown by the solid red arrows in Figure 6a (also Supplementary material; Table 5). Stormwater drains were associated with filamentous algae, whilst macrograzers had a negative effect on filamentous growth (Figure 6a). In contrast, macrograzers had a positive effect on foliose recruitment. Foliose cover was negatively affected by marinas, as were mesograzers (Fig 6b; supplementary material; Table 6).

Recruited leathery algal cover was positively influenced by sewerage outfalls, but not by the other impacts (Figure 6c, supplementary material; Table 7). The strongest relationship for this model was the negative influence of marinas on mesograzers. Sewage and fish farm inputs had positive effects on the total cover of recruited algae. Marinas were strongly positively associated with macrograzers (Figure 6d, Supplementary material; Table 8).

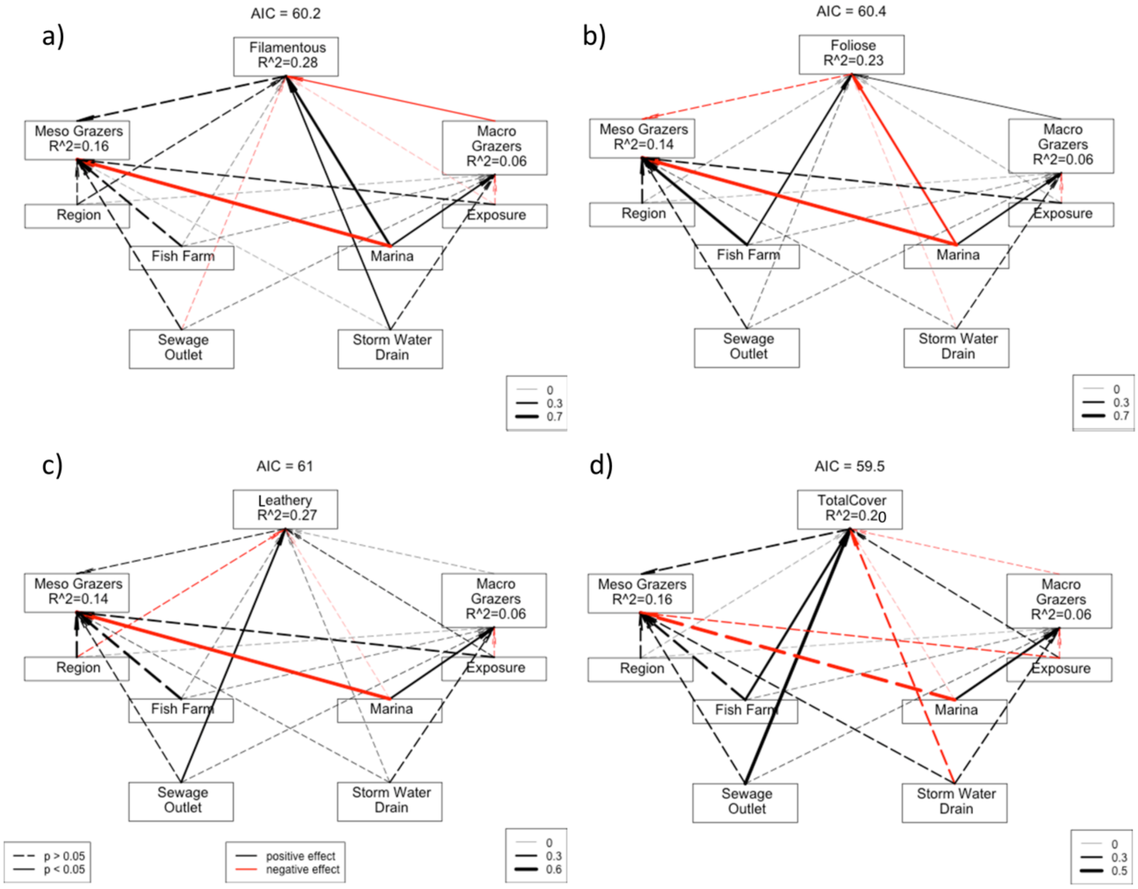

Figure 6. Structural equation models relating environmental and biological variables to the cover of recruited algal groups: a) filamentous algae; b) foliose algae; c) canopy algae; d) Total algal cover. Black arrows represent significant positive pathways, while red arrows represent significant negative pathways. Arrow thickness is scaled to match the standardized regression coefficients, with greater thickness indicating a stronger causal relationship.

Discussion

Urban pollution greatly affects marine benthic communities worldwide (Airoldi and Beck, 2007; Edgar et al., 2000; Vitousek et al., 2007). However, what we know of these impacts is often gleaned from studying individual components of the ecosystem separately and their response to one type of pollution in small-scale experiments. Our study examined the effects of common pollution sources on multiple trophic levels at the whole-estuary scale. We found that when total algae and grazers are considered separately, marinas have greatest impacts on transplanted communities and establishment of new recruits to empty space.

Mesograzers were negatively influenced by marinas, and this did not appear to be related to a change in the palatability of algae. When this information was integrated together in a network analysis with SEMs, there is little support for key indirect mechanisms, with pollution types exerting stronger direct influences on cover of different algal groups than indirectly though impacts on mesograzers and macrograzers. This suggests results of previous chapters, where grazers and indirect effects were not considered, were unlikely to be confounded by unknown ecological interactions involving grazers, and that patterns observed were likely primarily due to direct pollution impacts on sessile communities.

Influence of common pollutant sources on algal and grazer groups

Total cover of algal recruits increased at fish farms and near sewerage outfalls, an outcome that agrees with previous investigations where primary producers have been shown to respond positively to increasing nutrient levels in shallow coastal oligotrophic waters (Schramm and Nienhuis, 1996). Algal recruits in our study included a high percentage of opportunistic species, which are characteristic of habitats with elevated nutrients (Murray and Littler 1978).

Total macrograzer abundance exhibited a trend for increase under urban impacts, whilst total mesograzers were more sensitive to pollution, as evident in other studies (De-la-Ossa-Carretero et al., 2012; Edgar and Barrett, 2000). Macrograzers considered here included sea urchins, gastropods. In chapter 2 we found different trends in sessile communities with pollution in Sydney and Melbourne that appeared to clearly relate to sea urchin densities

(Stuart-Smith et al 2015). Thus, although our SEM analyses suggest pollution impacts the sessile communities more through direct than indirect pathways, some key macrograzers such as sea urchins are pollution tolerant, and thus show that alternative (non-pollution) drivers of macrograzer densities can indirectly affect sessile community structure and may over-ride pollution impacts.

In the Derwent Estuary, stormwater is the second largest source of heavy metals (Coughanowr and Whitehead, 2013), which can greatly affect mesograzer numbers (Ramos-Gómez et al., 2009). Such declines can result from behavioural avoidance as well as direct mortality (Roberts et al., 2008). The loss of mesograzers near marinas could influence the effects of epiphytes on nearby algae, with implications for restoration strategies. No other study has examined the response of mesograzers to marina impacts. Future research on grazer functional roles, and resilience provided by this group is certainly needed.

Is pollution changing the palatability of algal habitats?

The palatability of algal communities can be influenced by pollutants and environmental conditions through changes in species composition (and associated average palatability of species present) or through changing productivity, with concomitant change to their quality as food (e.g. C : N ratio) (Gaines and Lubchenco, 1982; Falkenberg et al., 2013; Nielsen, 2004). Altered palatability can thus change the degree to which grazers mediate indirect effects of pollution. Reduced grazing rates have been shown in amphipods on Cu-contaminated *Sargassum*, for example (Roberts et al. 2006).

Our measure of palatability was based on Poore's (2012) global study of grazing impact on algal morphology, and provided only an estimate of changes associated with variation in species composition, rather than including within-species chemical or morphological changes. The mean palatability of transplanted communities at urban pollution types did not differ significantly from those at control sites, suggesting interactions between the loss of established species and grazing rates were unlikely to be mediated by changed palatability. Nutrient pollution from fish farms, however, was associated with increased mean palatability of recruiting algal species. Thus, the composition of new recruits was biased towards those species which are more highly palatable at these elevated nutrient impact sites in comparison to the nearby controls. The positive relationships between sewerage and fish farms with mesograzers, and between the types of algae recruiting with mesograzers in our SEMs (albeit mostly non-significant), presumably in part reflect such improvements in food sources at these impact sites.

Effects of pollution on trophic pathways

Structural equation models (SEMs) indicated that pollution, rather than grazers, directly influenced established algal cover in temperate urban estuaries. Importantly, there was also a positive pathway from canopy algae to mesograzers, which agrees with observations that this algal group supports high densities of small grazers who have important ecological roles (Poore 2009). Loss of this group therefore has indirect effects on biota.

Marinas generally exerted the strongest influences within the SEMs, directly driving the responses of the algal functional groups, and in some cases also significantly affecting grazers. Marinas were consistently associated with high levels of abundance of total macrograzers; which may be related to local shelter and a locally higher abundance of invertebrates near wharves/marinas (Ling et al., 2012). Total algal cover and mesograzers were strongly negatively affected by marinas, a likely consequence of effects of contaminants (Piola et al., 2009, Birch and Taylor, 1999). Copper and Tributyl tin (TBT) has been widely used as the active biocide in antifoulant paint for the hulls of vessels for several decades in the Derwent region. In 2008, the application, re-application, or use of harmful anti-fouling systems containing TBT was prohibited in Australia (AMSA, 2016), however TBT accumulates over time in sediments in particularly high concentrations in areas of runoff from boat wash-down areas and under marinas and wharves (Scott 1993). The release of this toxic agent can prevent reproduction, particularly in neogastropods (Ellis & Pattisina 1990, Stewart et al. 1992). In addition, sediments from boat disturbance may also reduce macroalgal productivity, either by smothering of algal recruits or through reduced light from increased turbidity in the water column (Lyngby and Mortenson 1996, Cheshire et al. 1999).

Fish farms had a consistent positive effect on mesograzers, which may be explained by an increased abundance of opportunistic algal species, creating available food and habitat.

Habitat complexity thus appears to play an important role in influencing the assemblage structure of marine fauna, supporting earlier work (Edgar, 1992, Ayala and Martín, 2003). Fish farms had a positive influence on foliose recruits, a result consistent with other studies showing farm facilitate fast growing algal species (Munda and Veber, 2004; Oh et al., 2015). Total algal cover and leathery algae were also strongly positively affected by potential nutrient inputs from sewerage outfalls and fish farms, although perennial species can colonize poorly in nutrient-enriched waters (Korpinen et al., 2007). Fish farms also exerted a strong positive effect on the abundance of mesograzers, which may capitalize on conditions conducive for algal growth. This finding aligns with records of mesograzer outbreaks near aquaculture facilities (Brawley and Xiugeng, 1987), and also illustrates the sensitivity of estuarine ecosystems to nutrient inputs.

Overall, relationships identified in the algal recruitment SEMs were similar to the transplant SEMS, but with recruits more sensitive to grazer interactions. Propagules and germlings are delicate, often lacking mechanisms of protection or resistance against physical and biological stresses found in adults (Lubchenco 1983; Brawley and Johnson1 991). Thus directions of arrows were similar in both sets of SEM, but with some difference in statistically significant pathways.

We found little influence of either macro- or mesograzers on transplanted rocky reef habitat in the Derwent Estuary. A strong bottom-up effect in the Derwent Estuary contrasts control of mesograzers on seagrass epiphytes (Heck & Valentine2006), periphyton in freshwater systems (Hillebrand 2008), and algal epiphytes growing on coralline algal turf (Berthelsen and Taylor, 2014). But there are also many studies that have found either no variation in top-down control (Borer et al.2005; Hillebrand 2009; Poore et al 2009), or declines in marine grazer impacts (Burkepile & Hay 2006) with increasing ecosystem productivity. Likewise, macrograzer control of sessile communities is known to be important in many systems, including contributing to contrasting patterns in Sydney and Melbourne where the impacts of sea urchins are seemingly not controlled directly by pollution (discussed above). Our study thus highlights the context dependence of meso and macrograzer impacts on sessile communities, and likely importance of higher trophic levels (such as predators of grazers) or broader-scale environmental variation, among other factors not considered in this study.

Further research is needed to assess the importance of other important biotic and abiotic drivers, such as seasonal variability, light levels, wind resuspension, and water depth. The models presented here are simplifications of real food webs, and focus on particular steps we hypothesised to be important: pollution impacts influencing algae, algae influencing mesograzers, and macrograzers consuming primary producers. Observed variations relating to multiple human activities in estuarine food webs are clearly much more complex. While most observed relationships were consistent with prior studies, some paths were unclear, perhaps reflecting inadequacies in the dataset, non-linear relationships, or missing trophic paths. Moreover, all these processes are additionally influenced by the environment and traits of both macroalgae and grazers. Indeed, we acknowledge the difficulties in assessing complex multispecies systems, complicated further by multiple and interacting environmental and anthropogenic stressors. Regardless, our study comprises an advance as it adopts a more system-wide approach to the study of human impacts on natural ecosystems, and in turn generated additional hypothesises for future testing.

Acknowledgements

We thank the many students and volunteers who assisted with field and laboratory research, particularly Tania Mendo, Luis Henriquez, Lara Marcus. Thanks to Simon Grove of the Tasmanian Museum and Art Gallery for assistance with identifying gastropod species. Thanks to Justin Hulls for assistance with GIS calculations and Anna Cresswell and Jon Lefcheck for advice on structural equation modelling.

Appendix

TRANSPLANT

Table 1. Filamentous: Comparison of standardized regression coefficients (+/- standard error) for predictor environmental and pollution impacts for biological variables**.** Crosses (†) indicate log_{10}-transformed variables. Significant value are in bold. * P < 0.05, ** P < 0.01.

	Response		
Predictor	**Mesograzer abundance†**	**Filamentous algae**	**Macrograzer abundance†**
Fish farms	0.46 ± 0.23	**0.19 ± 0.09***	0.06 ± 0.18
Marinas	-0.57 ± 0.29	**0.45 ± 0.08****	**0.31 ± 0.16***
Sewerage	0.24 ± 0.26	-0.11 ± 0.08	0.08 ± 0.17
Storm water	0.09 ± 0.24	**0.23 ± 0.08****	0.21 ± 0.16
Upper estuary	0.32 ± 0.35	**0.34 ± 0.07****	0.02 ± 0.15
Exposure	0.29 ± 0.34	**0.28 ± 0.10****	-0.08 ± 0.21
Filamentous	-0.03 ± 0.28	-	
Macro grazer		-0.07 ± 0.06	

Table 2. Foliose: Comparison of standardized regression coefficients (+/- standard error) for predictor environmental and pollution impacts for biological variables. Crosses (†) indicate log_{10}-transformed variables. Significant value are bolded. * P < 0.05, ** P < 0.01.

	Response		
Predictor	**Mesograzer abundance†**	**Foliose algae**	**Macrograzer abundance†**
Fish farms	**0.54 ± 0.23***	0.05 ± 0.08	0.06 ± 0.18
Marinas	**-0.59 ± 0.26***	**-0.26 ± 0.07****	**0.32 ± 0.16***
Sewerage	0.29 ± 0.26	-0.05 ± 0.07	0.08 ± 0.18
Storm water	0.11 ± 0.23	-0.00 ± 0.07	0.21 ± 0.16
Upper estuary	0.33 ± 0.34	**0.21 ± 0.07***	0.02 ± 0.15
Exposure	0.27 ± 0.32	0.01 ± 0.09	-0.08 ± 0.21
Filamentous	-0.62 ± 0.39	-	-
Macro grazer	-	0.07 ± 0.05	-

Table 3. Leathery: Comparison of standardized regression coefficients (+/- standard error) for predictor environmental and pollution impacts for biological variables. Crosses (†) indicate log_{10}-transformed variables. Significant value are bolded. * P < 0.05, ** P < 0.01.

	Response		
Predictor	**Mesograzer abundance†**	**Leathery algae**	**Macrograzer abundance†**
Fish farms	**0.47 ± 0.22***	-0.08 ± 0.11	0.06 ± 0.18
Marinas	-0.50 ± 0.26	-0.20 ± 0.11	**0.32 ± 0.16***
Sewerage	0.23 ± 0.25	0.12 ± 0.11	0.08 ± 0.18
Storm water	0.10 ± 0.22	-0.07 ± 0.11	0.21 ± 0.16
Upper estuary	0.70 ± 0.37	**-0.62 ± 0.10****	0.02 ± 0.15
Exposure	0.48 ± 0.32	**-0.41 ± 0.13****	-0.08 ± 0.21
Leathery	**0.59 ± 0.21****		
Macro grazer		-0.07 ± 0.07	

Table 4. Total algal cover: Comparison of standardized regression coefficients (+/- standard error) for predictor environmental and pollution impacts for biological variables. Crosses (†) indicate log_{10}-transformed variables. Significant value are bolded. * P < 0.05, ** P < 0.01.

		Response	
Predictor	**Mesograzer abundance†**	**Total cover**	**Macrograzer abundance†**
Fish farms	**0.47 ± 0.23***	-0.09 ± 0.11	0.06 ± 0.18
Marinas	-0.40 ± 0.27	**-0.43 ± 0.11****	**0.32 ± 0.16***
Sewerage	0.21 ± 0.26	0.20 ± 0.12	0.08 ± 0.18
Storm water	0.19 ± 0.23	-0.08 ± 0.11	0.21 ± 0.16
Upper estuary	0.39 ± 0.32	-0.16 ± 0.12	0.02 ± 0.15
Exposure	-0.07 ± 0.37	-0.07 ± 0.17	-0.08 ± 0.21
Total cover	0.39 ± 0.21		
Macro grazer		-0.11 ± 0.07	

<u>RECRUITS</u>

Table 5. Recruitment of filamentous algae: Comparison of standardized regression coefficients (+/- standard error) for predictor environmental and pollution impacts for biological variables. Crosses (†) indicate log_{10}-transformed variables. Significant value are bolded. * P < 0.05, ** P < 0.01.

		Response	
Predictor	**Mesograzer abundance†**	**Filamentous algae**	**Macrograzer abundance†**
Fish farms	0.40 ± 0.23	0.04 ± 0.13	0.06 ± 0.17
Marinas	**-0.68 ± 0.27***	**0.49 ± 0.12****	**0.32 ± 0.16***
Sewerage	0.27 ± 0.25	-0.06 ± 0.13	0.08 ± 0.17
Storm water	0.01 ± 0.23	**0.29 ± 0.13***	0.21 ± 0.16
Upper estuary	0.20 ± 0.34	0.21 ± 0.16	0.02 ± 0.15
Openness	0.27 ± 0.32	-0.01 ± 0.18	-0.08 ± 0.21
Filamentous	0.33 ± 0.20		
Macro grazer		**-0.21 ± 0.08***	

Table 6. Recruitment of foliose algae: Comparison of standardized regression coefficients (+/- standard error) for predictor environmental and pollution impacts for biological variables. Crosses (†) indicate log_{10}-transformed variables. Significant value are bolded. * P < 0.05, ** P < 0.01.

		Response	
Predictor	**Mesograzer abundance†**	**Foliose algae**	**Macrograzer abundance†**
Fish farms	**0.53 ± 0.25***	**0.32 ± 0.10****	0.06 ± 0.18
Marinas	**-0.67 ± 0.29***	**-0.42 ± 0.09****	**0.32 ± 0.16***
Sewerage	0.27 ± 0.26	0.09 ± 0.11	0.08 ± 0.17
Storm water	0.10 ± 0.23	-0.02 ± 0.11	0.21 ± 0.16
Upper estuary	0.34 ± 0.34	0.05 ± 0.16	-0.02 ± 0.15
Exposure	0.29 ± 0.33	0.03 ± 0.16	-0.08 ± 0.21
Foliose	-0.19 ± 0.26		
Macro grazer		**0.13 ± 0.06***	

Table 7. Recruitment of leathery algae: Comparison of standardized regression coefficients (+/- standard error) for predictor environmental and pollution impacts for biological variables. Crosses (†) indicate log_{10}-transformed variables. Significant value are bolded. * P < 0.05, ** P < 0.01.

		Response	
Predictor	**Mesograzer abundance†**	**Leathery algae**	**Macrograzer abundance†**
Fish farms	0.44 ± 0.23	0.06 ± 0.08	0.06 ± 0.18
Marinas	**-0.59 ± 0.27***	-0.01 ± 0.07	**0.32 ± 0.16***
Sewerage	0.19 ± 0.30	**0.30 ± 0.08****	0.08 ± 0.18
Storm water	0.08 ± 0.23	0.06 ± 0.08	0.21 ± 0.16
Upper estuary	0.34 ± 0.34	-0.15 ± 0.09	0.02 ± 0.15
Exposure	0.28 ± 0.33	0.12 ± 0.11	-0.08 ± 0.21
Leathery	0.15 ± 0.37		
Macro grazer		0.03 ± 0.05	

Table 8. Recruitment of total algal cover: Comparison of standardized regression coefficients (+/- standard error) for predictor environmental and pollution impacts for biological variables. Crosses (†) indicate log_{10}-transformed variables. Significant value are bolded. * P < 0.05, ** P < 0.01.

		Response	
Predictor	**Mesograzer abundance†**	**Total recruit cover**	**Macrograzer abundance†**
Fish farms	0.34 ± 0.25	**0.33 ± 0.13***	0.06 ± 0.18
Marinas	-0.49 ± 0.27	-0.02 ± 0.13	**0.32 ± 0.16***
Sewerage	0.21 ± 0.27	**0.48 ± 0.15****	0.08 ± 0.18
Storm water	0.20 ± 0.24	-0.28 ± 0.15	0.21 ± 0.16
Upper estuary	0.23 ± 0.32	0.03 ± 0.23	0.02 ± 0.15
Exposure	0.17 ± 0.26	0.17 ± 0.26	-0.08 ± 0.21
Total recruit cover	0.24 ± 0.20		
Macro grazer		-0.06 ± 0.08	

Chapter 6. General discussion

Macroalgal and sessile invertebrate communities dominate near-shore rocky reefs of temperate waters, providing habitat in which a diverse array of species can live and interact (Schiel and Foster, 2015). Reduction of important canopy species in some areas along urbanised coastlines has triggered debate about causes of losses and ways to restore lost habitat. The principal aims of the present thesis were to understand the effects of common urban impacts on rocky reef habitat in estuaries, and conditions and mechanisms that lead to habitat changes, with the intent of predicting or preventing future losses.

The results clearly support the consensus that anthropogenic stress, particularly in the form of urban development, exerts strong effects on rocky reef communities (Airoldi and Beck, 2007; Connell et al., 2008). Habitat loss was particularly severe near heavily polluted areas, where macroalgal communities were presumably less resilient than at more remote areas. Urban pollution sources also affect algal recruitment, favouring invasive and opportunistic species, and imposing stronger control on what recruits to bare substrate and establishes, than grazing by meso- and macrograzers.

Indicators

The composition of macroalgal species differs considerably across the broader region studied in this thesis, and therefore commonalities in urban impacts among cities identified in chapter 2, and also between the Derwent and D'Entrecasteaux Channel in chapters 3, 4 and 5, are most clearly related to traits of the macroalgal species present rather than species identity. Characteristics such as the cover of tolerant and opportunistic species were shared by communities under multiple types of urban stressors. These can be regarded as indicators of undesirable ecosystem change (Hewitt et al., 2005) that are apparently broadly associated with multiple urban impact sources across estuaries and regions.

While it is the tolerant and opportunistic species that become dominant components of the community in impacted areas, losses of sensitive species are also important from the perspective of biodiversity conservation (Benedetti-Cecchi et al., 2001). Reductions in the cover of Fucales and red foliose algae are of particular importance because of their role in forming complex habitat structure and in supporting high species richness, including some local endemic taxa. Loss of these algae is likely to be one of the first detectable indications of reduced estuarine health and may signal irreversible changes in underlying ecological processes (Scheffer et al., 2009).

Identification of indicators for specific types of pollution has proved difficult, however, even with a carefully designed field experiment. A much stronger signal was observed between the upper Derwent Estuary, where many different pollution sources are present and interacting, and the less impacted lower estuary, than between any of the specific pollution sources in the outer estuary (Chapter 3). A clear sign of the severity of cumulative impacts in urban areas, this result also implied that identification of indicators for different types of pollution sources will require coverage of many different estuaries, including ideally those where only a single type of pollution is present.

Restoration

Reef transplants can be used to restore complex reef communities in urbanised estuaries (Campbell et al., 2014). In appropriate circumstances, they can enhance the recovery potential of locally threatened populations (Perkol-Finkel et al., 2012) and have a positive effect on densities of mobile pelagic and benthic organisms (Anderson et al., 1997; Bologna and Steneck, 1993; Levin, 1994). However, three key hurdles need to be considered and overcome for ecosystems to return to a healthy state: current pollution loadings, historical contamination, and the reversal of alternative stable states.

Current pollution levels near capital cites still need to be improved dramatically for diverse canopy forming species to survive. The transplant experiment in chapter 3 demonstrated that current biotic and biophysical conditions (pollution, habitat quality) in the Derwent Estuary are not suitable to allow the natural ecosystem to recover from multiple stressors of human origin (Jackson et al., 2001; Lotze et al., 2006). This is likely to be the case in most urban estuaries, and highlights the need for scoping studies prior to committing to restoration.

Multiple legacy and contemporary pollution sources close to cities appear to exert much stronger influences than individual impacts, with persistence through the long-term. For example, the heavy metal contamination in the Derwent that occurred before 1980 (Bloom and Ayling, 1977) provides a likely explanation for the current absence of canopy species, and ongoing inhibition of natural recovery. Although most urban impacts have localized effects, cumulative impacts of pollution can lead to major changes in water quality over broader scales (Bonsdorff et al., 1997). The legacy of metals in the sediments, in combination with interactions between types of disturbance, may act to erode resilience of desirable reef habitat, intensify nonlinear responses of ecosystems to human impacts, and limiting their adaptive capacity (Crain et al., 2008).

In highly polluted areas, reefs subjected to several sources of pollution were notably less resilient than those under less stress. This suggests that resilience has been eroded and algal communities have transformed to an alternative stable state that will not soon recover without intervention. Once a community has moved to an alternative stable state, negative feedbacks can make it difficult for natural communities to regain a foothold. Even though pollution regulations and water quality may have improved, transplantation of reef communities may still be necessary to get the system past hysteresis that keeps it in an undesirable state. In heavily urbanised areas, strong biological interactions may also exist involving grazers and dominant turf-engineering species (Bellgrove et al., 2010), as is perhaps the case for urchins in Sydney Harbour (Chapter 2).

It is much harder to restore areas which have already shifted to an alternative stable state than to recover systems in the process of change (Folke et al., 2004). Studies on other marine systems suggest that diverse assemblages may have higher probability of recovery following perturbations than depleted assemblages (Perkol-Finkel and Airoldi, 2010). Likewise, the presence of particular key components of a healthy system may provide important feedback mechanisms. For example, *Ecklonia radiata* is generally more tolerant of pollution than other Laminariales, but less tolerant to grazing than the fucoid *Carpoglossum confluens*. Healthy *Ecklonia* can exert physical and biological control on its environment, by sweeping away detrimental sediments and triggering positive feedbacks that facilitate recruitment (Connell, 2005).

In addition, reefs in the lower estuary may be somewhat resilient to moderate pollution loadings because of the more dynamic environmental conditions that provide greater dispersion or diffusion of pollution impacts. This highlights the importance of considering environmental conditions and previous level of pollution impact when managing pollution loads. Aggregations of urban developments should be reduced as much as possible in low flow environments such as estuaries. When expanding into new areas, urban development should learn from past mistakes, and adopt improved technologies for dealing with urban wastes, recycling excess nutrients on land, and setting well-defined limits for impacts. Clearly, improved understanding of the current state of estuarine biodiversity and how to maintain or improve it is needed.

Cryptogenic and invasive species

A growing body of literature, supported by the results of chapter 4, indicates that important sources of pollution in urban estuaries can influence the establishment of sessile organisms on rocky reefs (Gorman et al., 2009). Many studies have highlighted the role of human disturbance in biological invasion (Johnston et al., 2009; Johnston et al., 2011; Johnston and Roberts, 2009; Lockwood et al., 2005). Propagule pressure of invasive species is high in estuaries where there is a high density of vectors (as a result of international shipping ports) (Floerl and Inglis, 2003; Ruiz et al., 1999). Invasive species increase on pontoons and pilings of marinas rather than at controls (Glasby, 1999).

Chapter 4 showed that non-indigenous species recruitment rates were disproportionally higher near sewerage outfalls and marinas. The establishment of non-indigenous organisms may contribute to ecosystem degradation and drive native species extirpation (Pyšek and Richardson, 2010), but may also contribute missing functional roles (Sousa et al., 2009). For example, pollution may decimate the native community, with invasive species then capitalising on empty niches, rather than the invasive species immigrating into a healthy community and outcompeting prior residents (Stuart-Smith et al 2015).

Grazing impacts in response to pollution

Pollution can stimulate a range of algal responses depending on pollution source, but rarely do experiments consider whether algae are impacted directly or effects are mediated indirectly through ecological interactions (Daleo et al., 2015; Duffy et al., 2015). We have contributed to the long running debate over bottom–up vs. top–down forcing in ecosystems by comparing pollution types, environmental parameters, and different algal forms on rocky reef across scales of tens of kilometres.

Chapter 5 shows that algal assemblages were directly affected by pollution impacts, whilst grazers had little influence on transplanted algae. On the other hand, recruited algae were more influenced by grazer interactions but this interaction remained weak, chiefly involving macrograzers positively affecting foliose cover and consuming filamentous algae. Pollutant stressors in estuaries, such as marinas, fish farms, stormwater drains, and sewerage outlets, had greater influences on algal groups, including an expansion of non-desirable algal species. Fish farm stressors seem to increase the palatability of algal recruits, making them more susceptible to grazers. Mesograzers were more sensitive to pollution than macrograzers, with negative responses to marinas but positive responses to fish farms and canopy algae density. Incorporating environmental factors and pollution impacts in our models helped resolve the relative strength of bottom–up and top–down forcing, and makes this study a more realistic representation of an estuarine ecosystems. Overall, the direct algal responses to pollution (bottom up processes) appeared to drive trophic and community structure in urban areas. The extent to which the results of this study relate to estuarine conditions globally may differ with respect to grazer control and interactions, but the identification of stronger direct influences of pollution suggests it will clearly be a primary driver of sessile community structure in estuaries worldwide.

Estuaries at risk of pollution impacts

Assessment of broad scale impacts of urban pollution on recruitment and survival of habitat-forming communities in estuaries is complex (Veríssimo et al., 2013). The patchiness of estuarine reefs results in a high level of local scale spatial variability, making anthropogenic changes in community difficult to distinguish from background natural variation. Regardless, significant changes in reef assemblages were identified in all chapters of this thesis, an indication of the magnitude of human-induced change.

Low wave action, slow residence times, low water mixing, inputs of fresh water, extreme water temperatures, nutrients, sedimentation, turbidity, low light conditions, and a variety of other factors all have the capacity to shape communities (Hill et al., 2010), creating a naturally stressed environment dominated by tolerant species. Many features of natural estuarine environments are also common to those found in anthropogenically-stressed areas (Elliott and Quintino, 2007). Although they are supposedly adapted to cope with stress and have been regarded as resilient (Elliott and Quintino, 2007). Reef in estuaries may be susceptible to long-term change because recovery can only happen through recruitment of larvae or algal propagules from unaffected populations nearby (Hawkins et al., 1999).

I speculate that the fragmentation of reef in estuaries and low dispersal ability of many reef species can influence the rate of recovery, even if water quality improves, because the potential colonists are not sufficiently close for reproductive propagules to settle and develop. Generally algal species are not good dispersers, settling onto substrates within hours or days (Phillips, 2001, 2013). Rhodophyta, Fucales and kelps produce large, non-flagellate reproductive cells that sink to the substrate quickly rather than being widely dispersed. Without abundant perennial propagules, reef communities can change very quickly to less desirable habitats.

Methods for monitoring and forecasting change

Pollution impacts and resulting shifts in community structure can be especially difficult to detect in estuaries due to the magnitude of natural environmental variation (Chapter 2). To overcome this, the present study used controlled experiments replicated across space, and microhabitats, allowing for the incorporation of environmental context into the analysis, and multiple techniques were used for inference, including observational data and experimental transplants of adult assemblages and recruitment to settlement plates. Data were assessed at the community level, as well as morpho-functional group classifications (Balata et al., 2011; Littler and Littler, 1984; Steneck and Dethier, 1994). The groupings in each chapter were based on classifications relevant to the hypotheses of that chapter. Advantages and disadvantages of each component are also acknowledged, including that recruitment data creates bias in favour of fast-recruiting taxa, preferentially sampling weedy species that may be more common in modified estuaries. Interactions with environmental factors were also considered in models (Chapters 2, 3, 4 and 5), to explicitly account for covariation in the spatial and natural environment. The conclusions of this thesis are thus based on a relatively robust combination of approaches that provides considerable weight of evidence.

Recommendations and future research

Reefs in estuaries are generally situated close to shore which has, in part, rendered them more susceptible to human impacts. However this also makes them easier to monitor and manage. I suggest that an acceptable level of impact allows a structurally complex reef to be self-sustainable, which will facilitate and maintain biodiversity (Graham, 2004). In terms of habitat complexity, K-strategists are often more desirable than r-strategists, indicating 'healthier' or less-stressed environments.

Interactions between different pollution types in estuaries can complicate biological community response and the assessment of targeted management strategies (Strain et al., 2015). These multiple impacts can be heightened by local environmental attributes, and must be excluded from ecologically sensitive areas (Rivero et al., 2013), such as sheltered, low-energy environments with little flushing (Ross et al., 2004). Reducing one or more pollution sources can benefit foundation species (Crain et al., 2008) and efficient management should focus on critically-important impacts such as marinas. In particular, a strong need exists to set benchmarks and manage biodiversity and 'health' in estuaries through tracking real change in biodiversity, rather than relying solely on monitoring of water-quality parameters.

Conclusion

Globally, estuaries are currently experiencing severe stress (Halpern et al., 2008). Urbanisation and industrialisation of these areas has led, via several parallel and interacting paths, to a large-scale decline in abundance of sessile benthic species, and has likely caused many local extinctions and potentially a number of global extinctions (McKinney and Lockwood, 1999; Edgar and Samson, 2004). Macroalgae in urban areas are often living close to their tolerance limits, resulting in an impoverished species pool with a low number of species compared to healthier locations. Possible flow-on effects of declining foundation species on estuarine ecosystem biodiversity are an increasing concern. This thesis used co-located data on pollutants and natural environmental stressors to clarify relationships between anthropogenic stressors within and across multiple estuaries. Tests for human impacts on estuarine sessile reef communities have rarely been conducted in detail at spatial scales as large as in this study.

In summary, this thesis presents evidence that: (1) estuaries have been negatively affected by heavy urbanisation, with a phase shift to simple algal forms, however, certain species may be able to adapt to pollution stress and considerable caution must be exercised in the selection of benthic algae as biological indicators of pollution; (2) translocation of resistant perennial macro-algae can assist recovery of estuarine reef ecosystems, but recovery will not occur unless water quality has been improved and legacy pollution is not too severe; (3) different pollution sources affect recruitment of sessile benthos in different ways, such as the increase of non-indigenous and opportunistic species; and (4) pollution effects can override ecological effects associated with grazing.

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